SCREENING PUTATIVE PATTERN RECOGNITION RECEPTORS INVOLVED IN YEAST PERCEPTION

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Tiivistelmä – Referent – Abstract

Hiivat ovat merkittäviä kasvipatogeeneja, mistä huolimatta kasvien ja hiivojen vuorovaikutukset ovat edelleen huonosti tunnettuja. Nisäkkäillä on todistettu olevan immuunireseptoreita hiivoille tunnusomaisille molekylaarisille geenialueille (MAMPs, microbe-associated molecular patterns) ja siten on ajateltu, että myös kasveilla on vastaavia reseptoreita. Kun nämä reseptorit (PRRs, pattern recognition receptors) tunnistavat vieraita molekyylejä, käynnistyy kasvin immuunijärjestelmä, johtaen vieraiden molekyylien aiheuttaman immuniteetin (PTI, pattern-triggered immunity) ensimmäiseen vaiheeseen. Hiivan soluseinä on kerrostunut, joten vain osa sen molekyyleistä, kuten mannaanit, ovat helposti kasvireseptoreiden tunnistettavissa. Hiivojen ja PR-reseptoreihin perustuvan hiivaresistenssin hyödyntämiseksi maa- ja metsäteollisuudessa on tärkeää tunnistaa kyseiset reseptorit ja ymmärtää paremmin niiden toimintaa kasvien ja hiivojen välisissä vuorovaikutuksissa.

PR-reseptorit voidaan jakaa kahteen ryhmään, reseptorin kaltaiset proteiinit (RLPs, receptor-like proteins) ja reseptorin kaltaiset kinaasit (RLKs, receptor-like kinases). Osan L-tyypin lektiini-reseptorikinaaseista on osoitettu osallistuvan kasvien ja sienten sekä sienten kaltaisten patogeenien vuorovaikutuksiin, tehden niistä lupaavia ehdokkaita hiivareseptoreiksi. G-tyypin lektiinit ovat vähemmän tutkittuja, mutta myös niiden joukossa on mahdollisia hiivareseptorigeenejä.

Tässä tutkielmassa käytettiin sekä suoran että käänteisgenetiikan (forward and reverse genetics) menetelmiä osoittamaan, että lektiini-reseptori-kinaasit saattavat sisältää mahdollisia hiivamolekyylejä tunnistavia reseptoreita. Tutkielmassa kehitettiin ensimmäinen menetelmä ja geneettinen seula, jonka avulla voidaan tunnistaa kasvien kanssa vuorovaikuttavien hiivojen soluseinän molekyylejä tunnistavia kasvireseptoreja. Tutkimuksessa keskityttiin niihin lektiinireseptorikinaasigeeneihin, jotka ovat läsnä sekä rauduskoivussa (*Betula pendula*) että lituruohossa (*Arabidopsis thaliana*), mahdollistaen jatkotutkimukset rauduskoivun ja dimorfisen koivupatogeenin *Taphrina betulina* vuorovaikutuksesta *Arabidopsis*-mallijärjestelmässä. Näin saatua tietoa voidaan myöhemmin hyödyntää kasvintuotannossa myös muilla kasveilla ja niiden hiivoilla.

Avainsanat — Nyckelord — Keywords

kasvipatologia, reseptori, lektiini, RLK, PRR, MAMP, hiiva

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ABSTRACT

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Tiivistelmä — Referat — Abstract

Yeasts are significant plant pathogens, yet plant-yeast interactions are poorly understood. Mammals have immune receptors for yeast-specific MAMPs (microbe-associated molecular patterns), suggesting that plants might have similar receptors as well. Detection of MAMPs by PRRs (pattern recognition receptors) triggers the plant immune system, leading to the first phase of PTI (pattern-triggered immunity). The yeast cell wall is layered, so only some of the potential MAMP molecules, like mannans, are easily available for the plant receptors. In order to utilize yeasts and PRR-based yeast resistance in agriculture and forestry, it is crucial to identify these receptors and gain a better understanding of their functions in plant-yeast interactions.

PRRs can be divided into two groups, RLPs (receptor-like proteins) and RLKs (receptor-like kinases). Some of the L-type lectin receptor kinases have already been shown to participate in the interactions between plants and fungi or fungal like pathogens, making them promising candidates for yeast receptors. G-type lectins remain less studied but have promising genes as well.

In this thesis, we use forward and reverse genetics methods to show that lectin receptor kinases might include putative yeast pattern recognition receptors. We also establish the first protocol and genetic screen for the identification of plant PRRs participating in the recognition of yeast cell wall MAMPs. Only genes present in both silver birch (*Betula pendula*) and arabidopsis (*Arabidopsis thaliana*) were examined to enable following studies with an *Arabidopsis* model system of the interactions between silver birch and the dimorphic birch pathogen *Taphrina betulina*. This information can later be used in plant production with other plants and their yeasts as well.

Avainsanat — Nyckelord — Keywords

Plant pathology, receptor, lectin, RLK, PRR, MAMP, yeast

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ABBREVIATIONS

BAK1	BRI1-Associated receptor Kinase 1 (SERK3)
BIK1	Botrytis-Induced Kinase 1
BKK1	Bak1-like 1 (SERK4)
CERK1	Chitin Elicitor Receptor Kinase 1
C-LecRK	Calcium-dependent Lectin Receptor-like Kinase
EFR	Elongation Factor tu (EF-Tu) receptor
elf18	Elongation Factor Tu epitope 18
EMS	Ethyl Methanesulfonate
ETI	Effector-Triggered Immunity
ETS	Effector-Triggered Susceptibility
FLS2	Flagellin Sensing 2
fls22	Flagellin Sensing 2 Epitope 22
G-LecRK	GNA-related Lectin Receptor-Like Kinase
HR	Hypersensitive Reaction
LecRK	Lectin Receptor-Like Kinase
L-LecRK	Legume-like Lectin Receptor-like Kinase
LRR	Leu-Rich Repeats
LysM	Lysin Motif
MAMP	Microbe-Associated Molecular Pattern
MES	2-(N-morpholino) ethanesulfonic acid
NLR	Nucleotide-binding oligomerization domain-like receptor (NB-LRR)
PRR	Pattern Recognition Receptor
PTI	Pattern-Triggered Immunity
RLCK	Receptor-Like Cytoplasmic Kinase
RLK	Receptor-Like Kinase
RLP	Receptor-Like Protein
ROS	Reactive Oxygen Species
R protein	Resistance protein
SERK	somatic embryogenesis receptor kinase
SOBIR1	Suppressor of BIR1 1

1 INTRODUCTION

Climate change, modification of natural environments and high human mobility increase the spread of fungal and fungus-like plant pathogens and at the same time, food production must keep up with the rapidly growing human population (Fisher et al. 2012). Yeasts are significant plant pathogens and important residents of the plant's phyllosphere (Agler et al. 2016). Even if it is known that mammals have immune receptors for yeast-specific MAMPs (microbe-associated molecular pattern), the plant pattern recognition receptor (PRR) system for yeasts remains unknown (Wang et al. 2016).

PRRs could be used as the base for broad-spectrum resistance in economically significant crops and trees by modifying the PRRs or their associated molecules present in the host plant or alternatively, by transferring different PRRs between species to gain resistance to specific pathogens (Lacombe et al. 2010).

Yeasts can also be beneficial for plant growth and immune defences (Agler et al. 2016). Manipulation of the host plant microbiome with yeasts for the plant's benefit by limiting the growth of pathogens and favouring beneficial micro-organisms (Agler et al. 2016) has the potential to reduce the need for chemical fertilization and pesticides, making agriculture ecologically more sustainable.

To fully harness the agricultural potential of yeasts, more research on the underlying yeast-plant interactions is needed. Therefore, developing quick and effective screening methods for putative yeast MAMP receptors is essential for further studies on the subject.

2 LITERATURE REVIEW

2.1 Pattern-triggered immunity

Plant defence mechanisms can be divided into nonhost resistance and host resistance (Mysore and Ryu 2004). The nonhost resistance can be visibly symptomless (Type I) or lead to a cell death by hypersensitive response (HR, Type II) (Mysore and Ryu 2004).

To invade the plant, a pathogen must overcome passive defence mechanisms such as cell wall and possible constitutively produced antimicrobial secondary metabolites (Mysore and Ryo 2004). The first basal defense mechanisms of pattern-triggered immunity (PTI) activate when the susceptible plant host detects MAMPs (Jones and Dangl 2006), also known as general elicitors. These include production of reactive oxygen species (ROS), induction of general defense-related genes and cell-wall reinforcement with callose (Gómez-Gómez et al. 1999). Plant defence mechanisms require energy and can cause growth inhibition in the roots, leaves and cotyledons of arabidopsis (*Arabidopsis thaliana*) (Gómez-Gómez et al. 1999), which can often be easily observed.

Some pathogens have evolved to have effectors that can inhibit these mechanisms leading to effector-triggered susceptibility (ETS). In return, plants have adapted and evolved resistance (R) genes that encode R proteins (Jones and Dangl 2006). The main class of R proteins is NB-LRRs (also known as NLRs), polymorphic intracellular receptor proteins with nucleotide-binding and leucine-rich repeat domains, which can detect either effectors (non-self) or more often their effects on the host cells (modified self) (Jones and Dangl 2006). R genes are the basis of the more specific host resistance. Recognition of effectors and modified self can lead to localized cell death, a hypersensitive response, that limits the spread of the pathogen in the plant tissues and results in effector-triggered immunity (ETI) (Jones and Dangl 2006), also known as gene-for-gene resistance or vertical resistance.

Defences leading to hypersensitive reponse are effective towards pathogens from several kingdoms and towards obligate biotrophs and hemibiotrophic pathogens that require living plant material, but they can not prevent necrotrophic pathogens (Jones and Dangl 2006). To avoid ETI, natural selection causes pathogens to either gain new effectors, lose the old ones or modify the existing effector genes recognised by the host (Jones and Dangl 2006). This four-step pattern (Figure 1) is tied to the plant-pathogen coevolution and is commonly known as the Zigzag model (Jones and Dangl 2006).

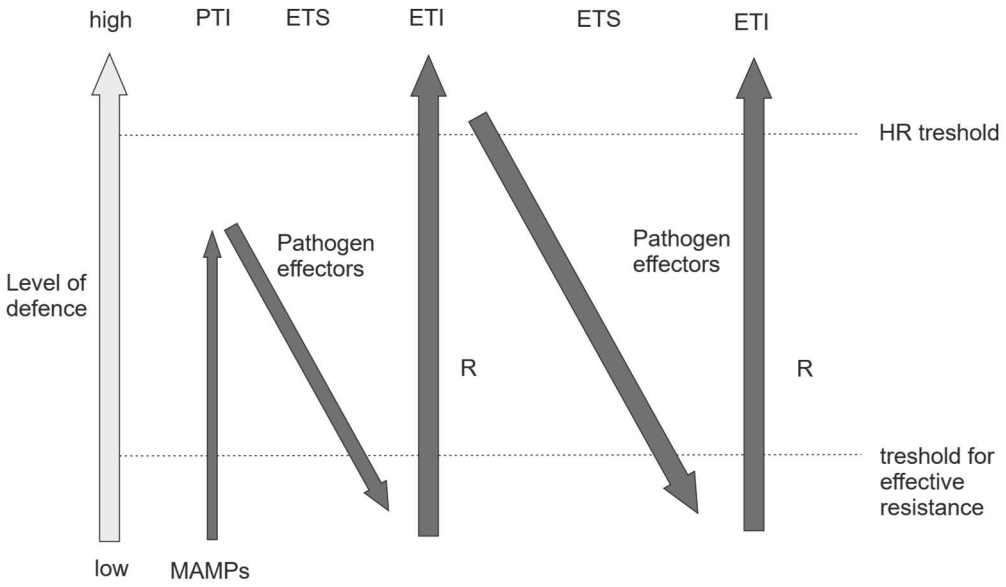

Figure 1. The Zigzag model depicting the evolutionary battle between the host plant and its pathogen. MAMP, microbe-associated molecular pattern; PTI, Pattern-triggered immunity; ETS, Effector-triggered susceptibility; ETI, Effector-triggered immunity; HR, hypersensitive reaction; R, recognition of pathogen effectors. Adapted from Jones and Dangl (2006).

2.2 Lectin receptor kinases

Pattern recognition receptors (PRRs) of the plant cell surface can detect different microbes from the microbe-associated molecular patterns (MAMPs) (Jones and Dangl 2006). Some MAMPs are recognised by several plant species, while some

are perceived only by a few different species (Kunze et al. 2004). For example, bacterial MAMP Ef-Tu is only recognised by the order *Brassicales* (Kunze et al. 2004). While R gene-based resistance can be easily evaded by pathogen evolution (Jones and Dangl 2006), PRRs are a part of the less specific nonhost resistance and they can recognise common, conserved microbial structures that are less likely abandoned by the microbes (Mysore and Ryo 2004). This makes them a promising candidate for broad and durable plant resistance.

PRRs are divided into receptor-like proteins (RLPs) and receptor-like kinases (RLKs) (Couto and Zipfel 2016). RLKs can be divided further into several subfamilies, one being lectin receptor kinases that are involved in plant development and respond to different stimuli, including biotic stress like fungi and fungal-like pathogens (Bouwmeester and Govers 2009, Mukherjee et al. 2010, Bouwmeester et al. 2011, Zhu et al. 2013, Wang et al. 2015).

Lectin domains can reversibly bind specific carbohydrate structures (Cassone et al. 1978). The different carbohydrate binding domains divide plant lectins into 12 families that include families like the Lysin Motif (LysM) family, the jacalin-related lectin (JRL) family, the legume lectin domain (L-type) family and the *Galanthus nivalis* L. agglutinin (GNA, G-type) family (Eggermont et al. 2017).

2.3 Yeast cell wall and MAMPs

Yeasts have a cell wall (Figure 2) that is composed of several potential MAMPs (Raacke et al. 2006). Some of these molecules are more readily available for the plant receptors, since the wall is layered and only a portion of these polysaccharides, such as mannans as mannoproteins and to some extend b1,6-glucans, are exposed in the cell surface (Cassone et al. 1978, Gopal et al. 1984, Klis et al. 2001).

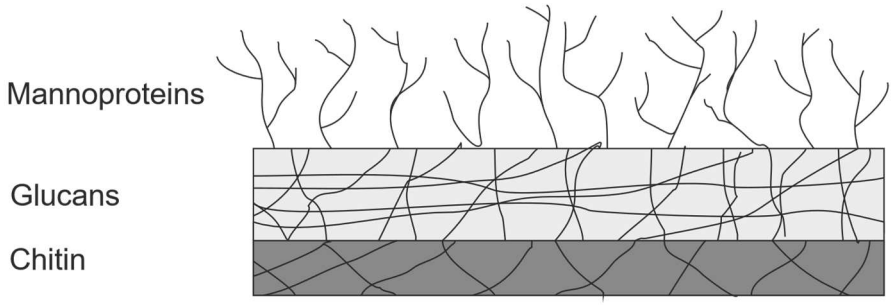

Figure 2. General structure of the yeast *Candida albicans* cell wall. Mannoproteins cover the outer yeast cell wall in a thick layer. The inner layer is comprised of a b1,6-glucan and b1,3-glucan network on top of the chitin network. Adapted from Perez-Carcia et al. 2011.

Chattaway and Holmes (1967) showed with the human pathogen *Candida albicans* responsible for candidiasis that the morphology of dimorphic fungi is determed by growing temperature and medium composition. Plant pathogenic dimorphic fungi have a life cycle that includes a haploid yeast phase that reproduces by budding and an often pathogenic dikaryotic or diploid hyphal phase (Sugiyama et al. 2006). *Ascomycota* subdivision *Taphrinomycotina* has a genus of dimorphic fungi *Protomyces* that includes plant pathogens able to infect plants families like *Apiaceae* and *Asteraceae* (Sugiyama et al. 2006). Another dimorphic fungal genus of the same subdivision is *Taphrina* including *Taphrina deformans* responsible for leaf curl on peach (*Prunus persica*) and the birch (genus *Betula*) pathogen *Taphrina betulina* causing witch's broom disease (Bacigálová 1997, Sugiyama et al. 2006).

Chitin is a common MAMP in fungal cell-walls (Cao et al. 2014). The amounts of fungal cell wall components vary between yeast and filamentous forms, filamental fungi having more chitin as shown by Chattaway and Holmes (1967) with *Candida albicans*. Plant LysM receptors are involved in chitin recognition and hence, LysM domain containing effectors are common in filamentous fungal pathogens, but not in human pathogenic yeasts (Kombrink and Thomma 2013) and dimorphic fungal plant pathogen *Protomyces spp.* (Wang et al. 2019) that inhabit plant phyllosphere as yeasts before transforming into hyphal form to infect their host.

Wang et al. (2019) suggest that this implies a lesser role for chitin in the recognition by the host plants.

In *C. albicans* the hypha cell walls have significantly more of the already abundant b1,6-glucan than yeast cell walls (Gopal et al. 1984). It is covalently linked to chitin and b1,3-glucan (Sietsma and Wessels 1981, Klis et al. 2001) and can be recognized by antibodies in yeast cells but not in hypha walls (Torosantucci et al. 2000). This suggests that it might be a good candidate to have a role in yeast-host interactions. The b1,3-glucans are also abundant in *C. albicans* yeast cell walls, but even more common in hypha cell walls (Gopal et al. 1984). The b1,3-glucans are normally buried under mannoproteins and mostly only exposed in budding scars (Klis et al. 2001, Gantner et al. 2005).

The cell wall composition can vary between different species (Bishop et al. 1960) but for example in baker´s yeast (*Saccharomyces cerevisiae*), mannans represent around 31 % of the chemical composition of the dried cell wall (Northcote and Horne 1952) and in the yeast form of *Candida albicans* 35-40 % of the cell wall polysaccharides (Klis et al. 2001), making them one of the most abundant molecules of the yeast cell wall (Northcote and Horne 1952, Cassone et al. 1978). Effector proteins with mannose linkage binding L-type lectin domains (Itin et al. 1996, Satoh et al. 2007) were found in all sequenced *Protomyces* genomes (Wang et al. 2019) and therefore, it can be hypothesized that mannose linkages and potentially other cell-wall carbohydrates could function as significant MAMPs in these interactions (Wang et al. 2019). Purified mannan is also relatively cheap and easy to acquire, making it ideal for the screening of the putative yeast receptor mutant lines.

2.4 Co-receptors and RLCKs

At the plant plasma membrane, PRRs and regulatory receptor kinases form dynamic heteromeric complexes that activate immune signalling and can later form multimeric complexes (Chinchilla et al. 2007, Somssich et al. 2015). In *Arabidopsis*, one of the most studied PRR is LRR-receptor kinase FLAGELLIN SENSING 2 (FLS2), which is known to interact with the co-receptor somatic

embryogenesis receptor kinase SERK3, also known as BAK1 (BRI1-ASSOCIATED RECEPTOR KINASE 1) (Zhang et al. 2009, Lu et al. 2010). Other SERKs also seem to form multimeric complexes with several other LRR-containing receptor-like kinases and RLPs (Couto and Zipfel 2016). On the other hand, LysM-containing PRRs interact with the co-receptor CHITIN ELICITOR RECEPTOR KINASE 1 (CERK1) (Cao et al. 2014).

SUPPRESSOR OF BIR1 1 (SOBIR1) is an RLK suppressor that is also known to interact with several immunity-related RLPs and has been suggested to transphosphorylate with BAK1 (Van der Burgh et al. 2019). Hence, it is a potential candidate possibly participating in plant-yeast interactions as well.

Another important component of PTI seem to be Receptor-like cytoplasmic kinases (RLCKs) (Zhang et al. 2009). The RLCK, BOTRYTIS-INDUCED KINASE 1 (BIK1), has been found to interact with FLS2 and BAK1 and potentially activate downstream signalling in the presence of flg22 (Chinchilla et al. 2007, Zhang et al. 2009, Lu et al. 2010).

2.5 Lectin receptor kinases, fungi and fungal-like pathogens

The role of lectin receptor kinases in plant-yeast interactions is yet unknown, but there are some studies with different fungi and fungal-like pathogens. Zhu et al. (2013) showed from RNA sequencing data that ascomycete *Fusarium oxysporum* effects the *Arabidopsis* gene expression levels for several genes, including AT1G65790 (SD17), At5g01540 (LecRK62) and At5g60900 (RLK1). Some *Arabidopsis* lectin domain proteins have also been indicated at the protein level to take part in the response to another ascomyceta, *Alternaria brassicicola* (Mukherjee et al. 2010).

Wang et al. (2015) showed that the *Arabidopsis* At5g10530 (LecRK91) and At5g65600 (LecRK92) mutants are less resistant to *Phytophthora* spp oomycetes and that they can also function independently of each other. Bouwmeester et al. (2011) have also shown that AT5G60300 (LecRK19) is an important component against the oomycete *Phytophthora brassicae*. *Phytophthora* spp. are known to

have small amounts of mannoproteins on their cell walls (Bartnicki-Garcia 1966) and it can be hypothesised that if mannans truly are important yeast MAMPs, the same receptor participating in the defense responses towards oomycetes could also be part of the immunity towards yeasts.

2.6 Forward and reverse genetics

Both phenotypic screens, forward- and reverse-genetic, have been used for receptors that can detect bacterial MAMPs, but not for receptors that participate in yeast-plant interactions. The basic principle is that defence mechanisms need energy, leaving less energy for the plant growth (Gómez-Gómez et al. 1999). Hence, the yeast receptor mutants can be longer and healthier than the wild type plants when grown with their specific MAMP, even if they do not exhibit any additional phenotypes when grown without the MAMP (Zipfel et al. 2006).

One of the most studied PRRs, LRR receptor-like kinase FLS2 (FLAGELLIN SENSING 2) was originally found by Lourdes Gómez-Gómez and Thomas Boller (2000) by using forward-genetic screen. In order to find growth-inhibited flagellin-insensitive mutants, they collected the normally growing EMS-mutagenized seedlings that were treated with bacterial epitope flg22 (Gómez-Gómez and Boller 2000).

Another well-studied PRR is EFR (EF-TU RECEPTOR), which was discovered by Zipfel et al. (2006) through a reverse-genetic screening of homozygous T-DNA-tagged LRR-RLK mutants. The five-day old mutants were grown on a MS medium with bacterial epitope elf18 (elongation factor Tu) for a week and the mutant lines that were growing better than the wild type were collected for further experiments. In this case, candidate genes to be tested were identified based on the patterns of gene expression in response to elf18.

3 RESEARCH OBJECTIVES

The aim of this study was to evaluate previously unknown yeast receptors present in the plant *Arabidopsis thaliana*. The goal was approached in two different steps:

I. Doing bioinformatics analysis of previously acquired *Betula pendula* and *Arabidopsis thaliana* data in order to find several lectin-domain genes as putative candidate yeast MAMP detecting PRRs.
II. Establishing and using laboratory screening methods like root assays to identify the genes in *Arabidopsis thaliana* that most likely participate in the recognition of carbohydrate groups common to the outer layers of the yeast cell wall.

4 MATERIALS AND METHODS

4.1 Plants

Most of the *Arabidopsis thaliana* mutants were acquired from the Nottingham Arabidopsis Stock Centre (NASC; http://arabidopsis.info/), but some were received from Doc. Kirk Overmyer (University of Helsinki, Finland), Kai Wang (University of Helsinki, Finland), Dr. Sachie Kimura (University of Helsinki, Finland), Assoc. Prof. Melinka Butenko (University of Oslo, Norway), Prof. Cyril Zipfel (The Sainsbury Laboratory, the United Kingdom), Prof. Klaas Bouwmeester (Wageningen University, the Netherlands) and Dr. Maija Sierla (University of Helsinki, Finland). The comprehensive list of all the lines used in this thesis, their origins and assigned names can be found from the appendices (Appendix 1).

All seeds were propagated before use in the experiments, the only exceptions being the Col-0 old line seeds that were used as such in some of the early experiments where the age of the seeds was not crucial for the results, as well as the yellow cameleon control seeds and M2 mutant seed pools that had been mutagenized by ethyl methanesulfonate (EMS) treatment of the background Col-

0 seed line that was transgenically expressing the calcium sensor system yellow cameleon under the control of a guard cell specific promoter (pCG1:YC3.6, Yang et al. 2008). These materials were obtained from Maija Sierla (Sierla et al. 2018). The information about the seeds used will be mentioned with each individual experiment.

4.2 MAMPs

The yeast extract used in the reverse and forward genetics experiments was Extrait autolytique de levure yeast extract (Lot 0006586, Biokar diagnostics, Beauvais Cedex, France). The mannan used for the reverse root assay plates was mannan from *Saccharomyces cerevisiae* prepared by alkaline extraction (Lot SLBT8710, Sigma-Aldrich, St. Louis, MO, USA).

4.3 TAIR chromosome map tool

Chromosome map showing the chromosomal localization of genes of interest were made of the four expanded gene families acquired from the OrthoMCL and Badirate results (Salojärvi et al. 2017 unpublished data). Aim was to see how closely the genes of each gene family are situated in regards of the other genes of that family, since closely situated genes can have same function, i.e. gene redundancy, and possible genetic linkage makes it hard to make double mutants or higher mutants, since the adjacent genes are often inherited together during the meiosis.

4.4 Genevestigator

The Genevestigator software (Zimmermann et al. 2004) was used to further narrow down the number of putative yeast-receptor candidate genes to test in the laboratory by accessing publicly available gene expression data to identify which transcripts were responsive to biotic stress in general. This was achieved by investigating how the fungus and fungal-like organisms available in the biological perturbations affected all the genes in the program. Affymetrix log2 with otherwise

default settings were used with p-values smaller than 0,05. If the gene was clearly down- or up-regulated, it was selected for further investigation.

4.5 The propagation of the plant seeds

The seeds were sown on 1:1 peat:vermiculite pots and vernalized in 4 ℃ for two days. After the vernalization the plants were transferred to controlled 12h/12h light cycle growth rooms (23 ℃ days/19 ℃ nights, constant relative humidity ~60 %, around 100-120 µmol/s/m2. The plants were grown for a week before transplanting to fresh pots.

After transplanting the plants were put to the same growth conditions for three more weeks before transferring to the greenhouse (18h light, 23 ℃, no humidiy regulation), were they were grown until the seeds were ready to collect.

The segregating mutant lines were genotyped by PCR using gene-specific primers (Appendix 2) to confirm homozygous plants from the segregating lines. Homozygous plants were identified from lines SALK_125442, SALK_024581 and SALK_146545 and their seeds were collected for further testing.

4.6 Establishing the pools for the forward genetics analysis

The A (pooled lectin RLK T-DNA KO mutants) and B (collection of known immune signaling mutants) pool mixes were made by counting 80 seeds from one line and comparing amount visually to an amount of seeds from the other lines to get an equal amount of approximately 80 seeds of each selected line to the mix. Only homozygous seeds were used. The EMS mutant pools 1-6 from Maija Sierla were used without further propagation. For further information, see Sierla et al. (2018) for a description of mutagenesis, pool structure, and other details of the production of M2 mutagenized seed pools.

4.7 Seed sterilization

Arabidopsis seeds were sterilized with 70 % EtOH and 2 % Triton X-100 (Batch 127K0018, Sigma-Aldrich Inc., St. Louis, MO, USA) solution for five minutes. The seeds were washed three times with 70 % EtOH.

4.8 Forward genetics assays

The sterilized seeds were first poured from an eppendorf tube to a sterile filter paper in order to ensure more even spread when pouring them on the agar plates. Pools A and B were tested to see if any seedlings would behave differently from others and grow bigger, suggesting that they might be blind to the yeast extract and the pools therefore likely contain resistant mutants for further testing. The A and B plates always had half of the plate covered with Col-0 seeds and the other half covered by either A or B seeds. Both had one 1,8 g yeast extract per liter plate and one 0,9 g yeast extract per liter plate.

These pools were formed from the seeds obtained from other scientists (B) and the seeds that were selected based on Genevestigator results and ordered from Arabidopsis stock centers (A). Both pools had a calculated amount of approximately four individuals per mutant line per plate and an equal amount of Col-0 seeds. Mutagenized seed pools (M2 generation) 1-6 (Sierla et al. 2018) were tested with col.B1 line and Yellow Cameleon (YC) pool controls.

The petri dishes with 0,5x MS agar without MES and 1,8 g/l yeast extract and their control plates without the yeast extract were incubated in the dark at 4 °C for two days and put horizontally in 12 h light (23 °C, 65 % humidity) / 12 h dark (18 °C, 75 % humidity) controlled growth chamber (Fitotron SCG120, Weiss Technik UK Ltd., Loughborough, Leicestershire, United Kingdom). The plates were visually inspected approximately every day except weekends to estimate, when would be the best time to choose the mutants for propagation. The plates were kept for ten days at most and potential yeast receptor mutants were collected under a stereo microscope.

Pools 1 and 2 were kept in same conditions but lower light intensity (~80-100 µmol/s/m2), which elongated the seedlings and made inspecting the plates

harder. Hence, the rest of the plates were always kept in ~120 µmol/s/m2. Pools 1 and 2 were sown on square 40 ml plates with 8000 seeds and a Col-0 control plate with the same yeast concentration and seed density to improve the efficiency of the assay.

Pools 3 and 4 were done the same way as pools 1 and 2 but for the pools 5 and 6 the seedling density was reduced by making two plates per pool that both had 2500 seeds. Yellow cameleon with the same density and amount of yeast extract as well as an agar plate without the yeast extract and covered in yellow cameleon seeds were used as controls. Pools 5 and 6 were sealed with parafilm only for the first two days in the light before changing the parafilm to medical tape in order to ensure better gas exchange.

4.9 Reverse genetics assays

Seeds were planted on 0,5 x MS agar medium containing 1 % (w/v) sucrose (Lot 17L064108, D(+)-saccharose, VWR International Ltd., Radnor, PA, USA), pH 5.6-5.8 with KOH, 0,8 % (w/v) agar (Lot SLVBV6049, A4550-500G, Sigma-Aldrich, St. Louis, MO, USA), 0,5 X MES (Lot 012078.07, MES monohydrate M1503.0250, Duchefa Biochemie B.V., Haarlem, The Neatherlands) and 0,5 X MS salts (Lot SLBN7853V, M5524-50L Murashige and Skoog Basal Salt Mixture (MS), Sigma-Aldrich Inc., St. Louis, MO, USA). Each concentration of yeast extract including mannan or pure mannan was added to the media it was autoclaved, but concentrated (6 g/100 ml) yeast extract water solution was autoclaved separately before adding it to the medium.

4.9.1 Initial assays

The initial concentration tests were performed with the non-propagated Col-0 batch 1 (col.B1) wild type seeds in order to find the ideal yeast concentrations for further testing. All experiments presented used the following plant growth conditions unless otherwise indicated. After sowing seed on the petri dishes with yeast extract were stratified by being incubated in the dark at 4 °C for two days to synchronize the germination of seeds. Plates were then put vertically at ~23

°C in 12 h light/12 h dark with ~80 µmol/s/m2 in a controlled growth room. The light conditions in the growth rooms and chambers were measured using a MQ-200 Quantum meter with separate sensor (Apogee instruments Inc., North Logan, UT, USA) and two sets of plates with yeast extract concentrations 0,9 g/l and 1,8 g/l were grown on two separate shelves of the growth room to confirm that the small changes in lightning and other environmental factors wouldn't have a significant effect on the results. After eight and 10 days the seedlings were photographed with a piece of millimeter paper as a size reference using Nikon D5100 (Nikon Inc., Tokyo, Japan). The non-germinated seeds were marked after four days in the light and left out of the final data. The root lengths were measured by ImageJ software (ver.1.52d for Windows 64-bit).

The yeast extract concentrations used were 3 g/l, 1,8 g/l, 0,9 g/l and 0,3 g/l. Each concentration was tested with 10-20 col.B1 wild type seeds per plate and repeated three times. Also, concentration 30 g/l was tested once, leading to inhibited agar solidification causing liquid media to leak out of the bottom of the plate because of the high amount of yeast extract. For this reason, experiments with this high concentration were discontinued. The initial concentration assays were performed with 0,05 % (w/v) MES in the agar.

According to Clara Sanchez-Rodrigues (the 29th International Conference on Arabidopsis Research 25.-29.6.2018 in Turku), buffers in the growth media reduces growth inhibition by elicitors since extracellular pH changes are important for the response. Based on this information, the effect of 0,05 % (w/v) of MES was tested against identical agar plates without MES on all four different concentrations and Col-0 seeds. Our results seemed to be in line with the results obtained by Sanchez-Rodrigues labratory, suggesting that the absence of buffer does allow a more robust inhibition of rooth length when the plant is grown on a MAMP containing media. Therefore, all further plate experiments with yeast extract and mannan were done without MES.

4.9.2 Testing co-receptors as potential positive controls

Lines *bkk1-1* (SERK4, BAK1-LIKE 1 mutant), *bak1-5* (SERK3, BRI1-ASSOCIATED RECEPTOR KINASE mutant) and *cerk1-2* (CHITIN ELICITOR RECEPTOR KINASE 1 mutant) and *bbc* (*bak1-5 bkk1-1 cerk1-2* triple mutant) were tested as possible positive controls and compared to the same age Col-0 batch 2 (col.B2) wild type line with yeast extract concentrations 3 g/l, 1,8 g/l, 0,9 g/l, 0,3 g/l and 0 g/l. The concentration 0 g/l and 0,9 g/l were selected for further studies with three replicates per line. Seeds were plated, grown and evaluated as described for the initial assays above.

4.9.3 Mannan assay

Three agar plates with the mannan concentrations of 0 g/l, 0,3 g/l and 0,9 g/l were made and lines *bbc*, *bkk1-1*, *bak1-5* and the same age col.B2 Col-0 wild type line seeds were sown on the plates, four seeds per line. Seeds were plated, grown and evaluated as the control mutant lines above.

4.9.4 Yeast extract assay with mutant lines and controls

The mutant lines and a possible positive control line *serk5-1* (Table 1) were tested with concentrations 0 g/l and 0,9 g/l with the col.B2 wild type seeds of the same age as negative control. Some genes had only one mutant line available and had therefore twice the amount of seeds than other lines.

Seeds were plated, grown and evaluated as before, except for growing the plants in a controlled growth chamber (Fitotron SCG120, Weiss Technik UK Ltd., Loughborough, Leicestershire, United Kingdom) with 12 h light (23 °C, 65 % humidity, ~120 µmol/s/m2) and 12 h dark (18 °C, 75 % humidity).

Table 1. Mutants tested with 0,9 g/l yeast extract (initial tests not included).

Gene	Mutant line	Assigned name	Position
At5g01540	VI 2-2 B	At5g01540.L1	unknown
At5g65600	SALK_111817C	At5g65600.L1	exon
	SALK_140495C	At5g65600.L2	exon
At5g60900	SALK_084958C	At5g60900.L1	exon
	SALK_146545C	At5g60900.L2	exon
At4g21390	SALK_099394C	At4g21390.L1	5'-UTR
	SALK_147351C	At4g21390.L2	intron
At2g37710	SALK_019496C	At2g37710.L1	exon
	SALK_069646C	At2g37710.L2	5'-UTR
At2g13800	serk 5-1	At2g13800.L1	unknown
At4g02420	SALK_128001C	At4g02420.L1	exon
At1g11330	SALK_143489C	At1g11330.L1	exon
At5g01550	SALK_108000C	At5g01550.L1	exon (and promoter of TE)
At1g11350	SALK_099776C	At1g11350.L1	exon
	SALK_026338C	At1g11350.L2	exon
At2g19130	SALK_000051C	At2g19130.L1	1000-promoter
At3g16030	SALK_136842C	At3g16030.L1	1000-promoter
At1g61610	SALK_201319C	At1g61610.L1	exon
At5g60280	SALK_147846C	At5g60280.L1	promoter
At4g04960	SALK_093876C	At4g04960.L1	exon
	SALK_051149C	At4g04960.L2	1000-promoter

L1 = line 1 acquired for this thesis, L2 = line 2 acquired for this thesis

4.10 Statistics

Depending on the data, either one-way ANOVA, two-way ANOVA for unbalanced design (Type III tests from the car package) or linear mixed model with repeat as a random effect (emmeans and nlme packages) was used to determ the

statistical significance of the results. Since the sample sizes in each data sample obtained vary between the different variables, two-way unbalanced design ANOVA was selected over the standard two-way ANOVA for balanced designs.

Tukey (TukeyHSD) with the confidence level of 95 % was used to get more detailed information of the significant differences, but it should be noted that this might not be ideal for unbalanced data. R documentation (www.rdocumentation.org) states that the function has an adjustment for mildly unbalanced designs, but all results should be approached with caution before more experiments and statistical tests have been performed.

ANOVA tests assume that the data is normally distributed, has equal variances and is independent and randomly observed. Normal distribution was verified by drawing a histogram and a normality plot of the residuals (data not shown). The homogeneity of variances was confirmed with the residuals versus fits plot (data not shown).

All analyses were performed using RStudio software (ver. 1.1.463, RStudio Inc., Boston, MA, USA).

5 RESULTS

5.1 Background

Lectin receptor-like kinases (RLKs) have a role in plant-fungal interactions (Eggermont et al. 2017) and were therefore selected as the main focus of this thesis. There are 200 RLK candidates (Eggermont et al. 2017) and 75 lectin receptor-like kinases identified by Vaid et al. (2012) that are one of the most promising RLK groups for yeast receptors. In order to narrow down the large gene sets, previous research results made by other researchers who collaborate with the plant stress research group were used as a starting point. As part of the silver birch (*Betula pendula*) genome sequencing and population genomics analysis (Salojärvi et al. 2017), Jarkko Salojärvi did a BadiRate analysis (Librado et al. 2012, Salojärvi et al. 2017 unpublished data) based on the OrthoMCL (Li et al.

2003) results by Sitaram Rajaraman (Salojärvi et al. 2017 unpublished data). BadiRate analysis shows gene families that are actively evolving through gene family expansion.

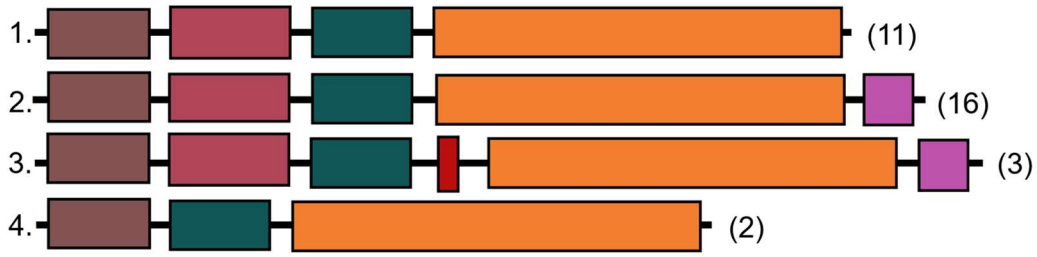

Figure 3. Expanded gene family one domain structures at superfamily domain level. Brown, G-type lectin; pink, S-locus glycoprotein; green, PAN/APPLE-like; orange, protein kinase; red, unknown function 3660; purple, unknown function 3403. Numbers in brackets indicate the number of genes with each domain structure found from *Arabidopsis*. Picture is based on the results acquired from NCBI Web CD-Search Tool and sequences used for the OrthoMCL.

The BadiRate analysis gave four of these expanded gene families (referred to here as expanded gene families one to four) with an adjusted p-value that was smaller than 0,05, so it is likely that all the expansion events have not happened randomly but rather because of a selection pressure, which could indicate a connection to pathogen resistance. The OrthoMCL results show which lectin receptor orthogroups that have undergone gene family expansions in the *Betula pendula* genome are also present in *Arabidopsis thaliana*.

Figure 4. Expanded gene family two structures at superfamily domain level. Blue, L-type lectin; orange, protein kinase. Number in brackets indicates the number of genes with similar domain structure found from *Arabidopsis*. Picture is based on the results acquired from NCBI Web CD-Search Tool and sequences used for the OrthoMCL.

In *Arabidopsis*, expanded gene family one had 32 genes (Figure 3), expanded gene family two had 33 genes (Figure 4), expanded gene family three had 1 gene (Figure 5) and expanded gene family four had 2 genes (Figure 6). These 68 genes present in both plants were selected as a starting point for this thesis. Genes in expanded gene families one and three belong to the G-type lectins, while the genes in expanded gene families two and four are all L-type lectins.

Figure 5. Expanded gene family three structures at superfamily domain level. Brown, G-type lectin; green, PAN/APPLE-like; orange, protein kinase. Number in brackets indicates the number of genes with similar domain structure found from *Arabidopsis*. Picture is based on the results acquired from NCBI Web CD-Search Tool and sequences used for the OrthoMCL.

In order to reduce the number of mutant lines further, Genevestigator (Zimmermann et al. 2004) was used to narrow down the genes to those either up- or down-regulated with fungal or fungus-like MAMPs and thus, more probable yeast resistance signalling candidates to test in the laboratory with the genetic screens developed here.

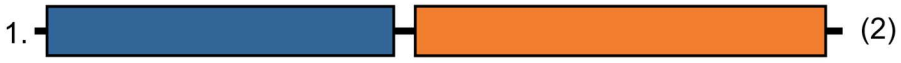

Figure 6. Expanded gene family four structures at superfamily domain level. Blue, L-type lectin; orange, protein kinase. Number in brackets indicates the number of genes with similar domain structure found from *Arabidopsis*. Picture is based on the results acquired from NCBI Web CD-Search Tool and sequences used for the OrthoMCL.

5.2 TAIR chromosome map tool

Many of the genes inside the expanded gene families one and two (Appendix 3, Figures 1 and 2) are situated close to each other, resulting in a high risk of gene redundancy, which could mask mutant phenotypes, and genetic linkage, which would prevent making double mutants in future studies. Families three and four (Appendix 3, Figures 3 and 4) are more favorable for screens based on phenotype.

Whenever possible, only single copy genes, or genes that only had a few copies that were not tandemly duplicated, were selected for the reverse genetics screening.

5.3 Genevestigator

Of the 48 genes tested with Genevestigator, 33 appeared to be either up- or downregulated in the presence of a fungus or a fungal-like MAMPs (Appendix 4). During this thesis, we were able to acquire 13 of these genotypes for testing in reverse genetic screens. Lines At5g65600, At5g60900, At4g21390, At2g37710 (LecRK41), At1g11350 (SD113) and At4g04960 (LecRK71) had two homozygous lines available, while lines At4g02420 (LecRK44), At1g11330, At5g01550 (LecRK63), At2g19130, At3g16030 (CES101), At1g61610, At5g60280 (LecRK18) had only one homozygous line to be tested.

5.4 Forward genetics assays

To see if the lines to be tested in the reverse genetics root assays included any putative yeast defence related mutants, all homozygous lines where first pooled and tested in bulk with the yeast extract at concentrations 0,9 g/l and 1,8 g/l. Both A and B pools clearly had several seedlings that seemed to be stunted when grown on the yeast extract (Figure 7). Compared to the Col-0 seedlings (Figure 8) the mutant pool plants appeared to suffer more from chlorosis and stunted growth, which could indicate that some of the plants in both pools are sensitive to the yeast MAMPs.

Some of the bigger mutant seedlings were relatively unaffected by yeast extract the same way as most of the Col-0 seedlings or appeared to grow even better than their Col-0 controls in the concentration 1,8 g/l. They might be mutants for a crucial plant defences against yeast and hence be blind to the yeast MAMPs. They can also be developmental mutants and need to be confirmed by sequencing and further testing. Chlorosis was less severe with the concentration 0,9 g/l (data not shown).

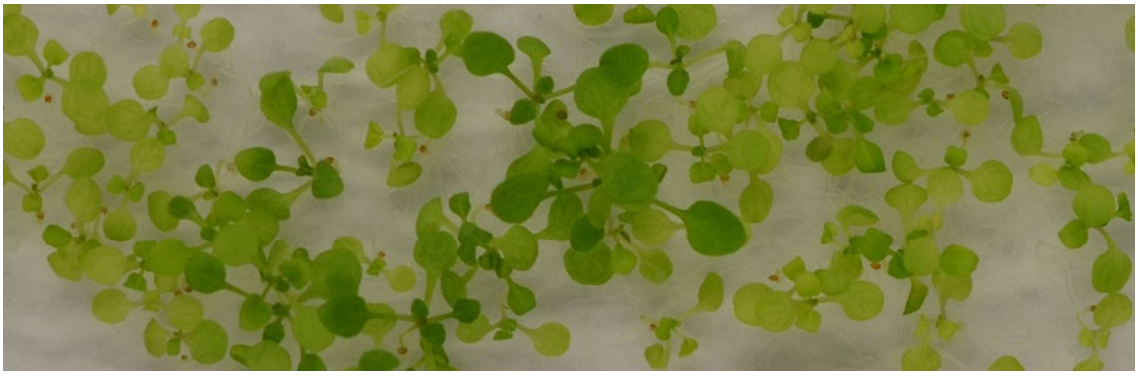

Figure 7. Forward genetics pool A (pooled lectin RLK t-DNA KO mutants) on a plate with 1,8 g/l of the yeast extract. Chlorosis and dwarfism were common for the mutants, yet some of them stood out by resembling the Col-0 control plants.

No mutants were collected from this initial test, but this experiment supported the hypothesis that both groups might have yeast-MAMP insensitive mutants and hence, potential yeast receptor candidates that should be tested further. This result encouraged further tests to try and identify the yeast-MAMP insensitive mutants.

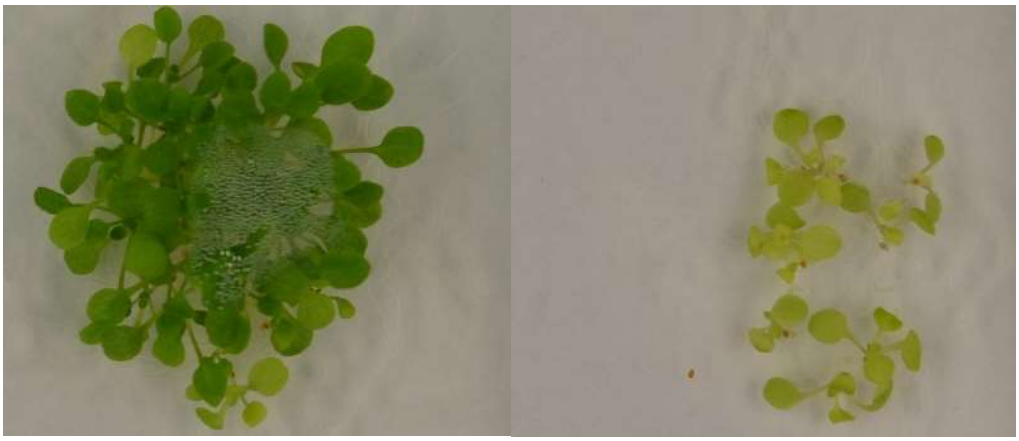

Figure 8. Forward genetics pool A (pooled lectin RLK t-DNA KO mutants) Col-0 col.B2 control plants on 1,8 g/l of yeast extract. Only some of the plants seemed to suffer from chlorosis and dwarfism while most looked green and healthy.

The seed density proved to be a real issue in the initial tests with pools 1-4 and 8000 seeds per plate. This potential for false positive mutants should be taken into consideration carefully in future experiments to improve the likelyhood of finding the resistant mutants with visual inspection. Lowering the amount of seeds to 2500 per plate made the inspection more pleasent, but also showed the importance of even distribution, so using a sieve or a grid of some kind during distribution could improve the method significantly.

Figure 9. Pool 6, plant 17. 2500 seeds on a plate. The mutants that were developmentally clearly further advanced of the others were collected from the EMS pool plates in order to find seedlings blind to the yeast extract.

Table 2. EMS pools with promising mutants and the amount of plants collected from each pool.

Pool	2	3	4	6
Mutants	1	7	7	3

All in all, 18 mutants were collected from the EMS pools (Table 2). The seedlings collected were developmentally further advanced of the other seedlings of the same plate (Figure 9) and might hence be yeast MAMP receptor mutants. For example, the seedling in figure 9 had seven leaves while most of the plants on the same plate as well as on the Col-0 control plate had, on average, four leaves. It is also possible that some of the collected mutants are developmental mutants unrelated to the yeast receptors, but their root system was not larger than that of the other plants and more experiments are needed to confirm the nature of the mutations.

5.5 Reverse genetics assays

If not otherwise stated, all datasets were tested for homogeneity of variance and normal distribution (data not shown). There was not enough proof to assume that these assumptions would have been violated enough to result in unvalid ANOVA tests, especially since all of the results should be considered preliminary and further repeated experiments are required in the future. The main purpose of this work was to establish screening conditions and identify putative mutants for future studies.

5.5.1 Initial assays

There appeared to be a clear difference in Col-0 root lengths between the different yeast extract concentrations and the control plants without any MAMPs (Figure 10). In order to establish reproducable conditions, the effect of different

yeast extract concentrations was tested to find the lowest concentration with a significant difference to the control plants.

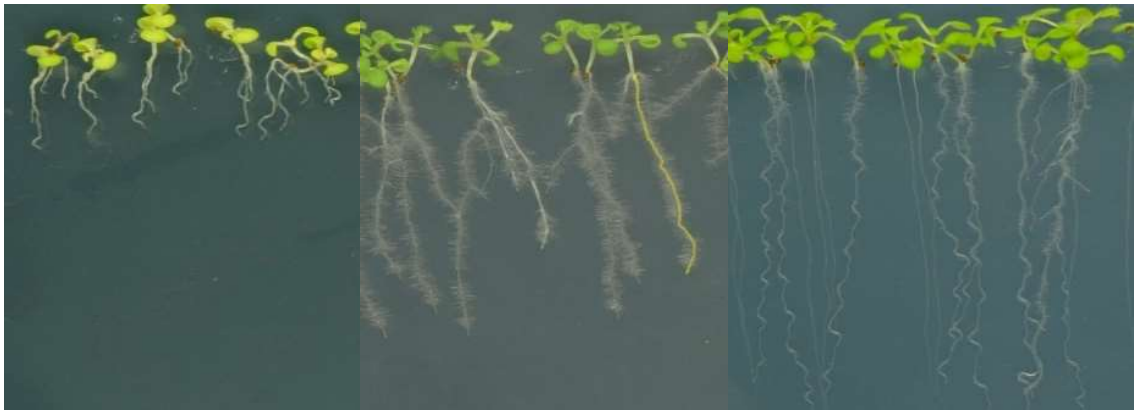

Figure 10. The *Arabidopsis* Col-0 root growth inhibition caused by different concentrations of yeast extract. The concentrations used from left to right where 3 g/l, 0,3 g/l and 0 g/l. The roots were measured with the ImageJ software by setting a scale from a known measurement and carefully measuring the length of the root as depicted in the picture in the middle with a plant grown with a 0,3 g/l concentration.

The treatment with different yeast concentrations is statistically significant at the confidence level 99,9 % (p-value <2e-16). ANOVA with Tukey post-hoc tests done to the original length data (Figure 11) suggests that there is a significant difference between the control plant lengths and the lengths of the other plants grown with different yeast extract concentrations, except with 0,3 g/l (p-value 0,8650045). Analysis done to the normalized (percentage) data (Figure 8), where the treated plant root lengths were divided by the average control lengths came to the same conclusion (p-values for 3 g/l and 1,8 g/l were both 0,0000000, for 0,9 g/l it was 0,0000076 and 0,1770182 for 0,3 g/l). This suggests that 0,9 g/l is the lowest yeast extract concentration tested able to induce a significant inhibition of root length compared to the media without yeast extract.

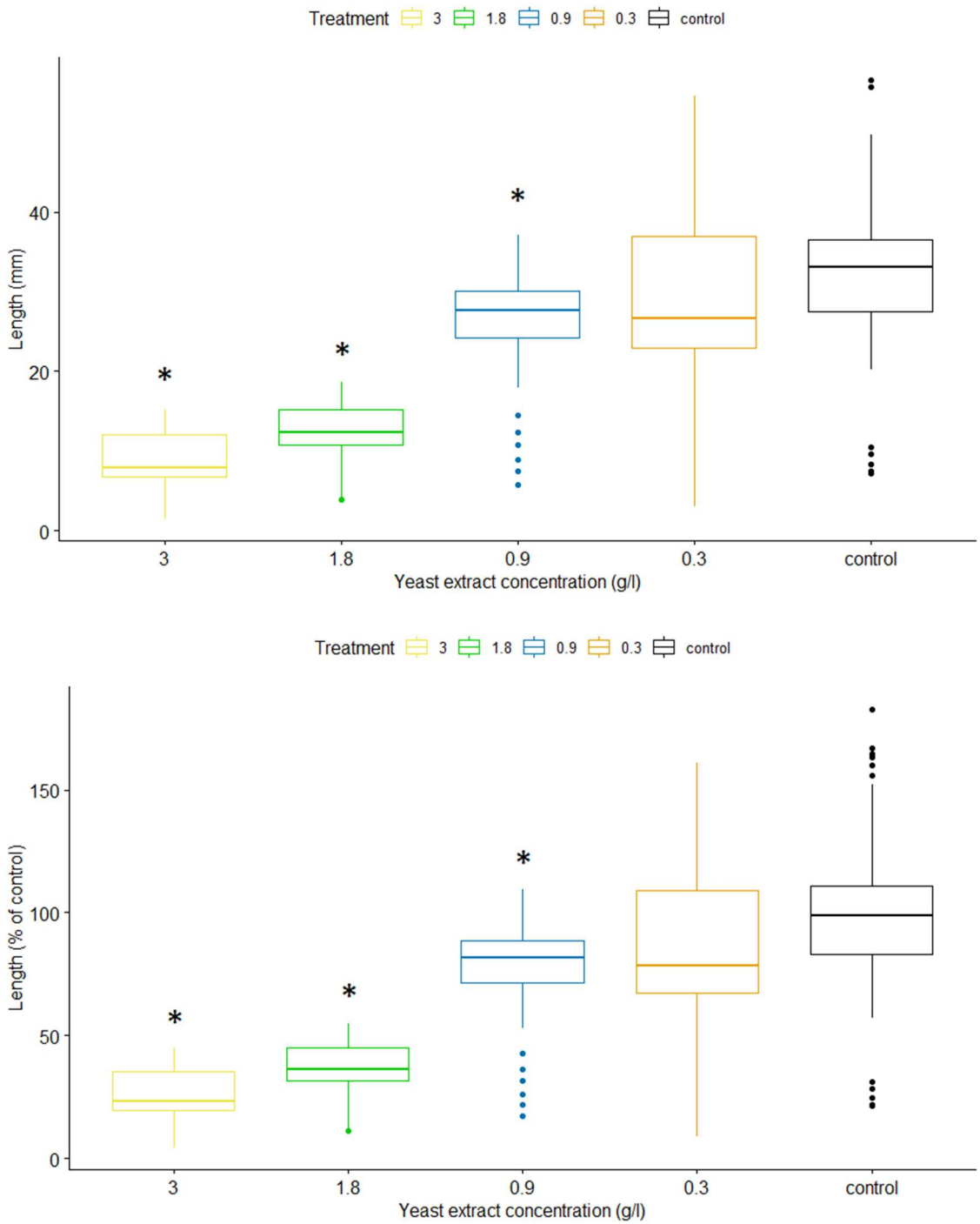

Figure 11. Top: Col-0 col.B1 root lengths with yeast extract on MES buffered media. Three biological repeats of all concentrations (N = 35-72); bottom: the same Col-0 col.B1 root lengths divided by the average control lengths shown as percentages (N = 35-54). Statistical tests used were one-way anova and Tukey multiple comparisons of means at the confidence level of 95 %.

There doesn't seem to be a noticable difference between the root lengths and the percentage results calculated by dividing the root lengths with the average control lengths (Figure 11) and therefore, only the percentage plots will be shown in the rest of the result section of this thesis. Normalizing the data with the controls of each plant line will remove the artifacts of possible developmental mutants that have longer or shorter root lengths under control conditions. It will also give more often the same N count (number of technical repeats within experiments) between the different groups. This makes the design more balanced for testing with ANOVA and Tukey since the control plants often had less germination problems and as a result, more seedlings to measure.

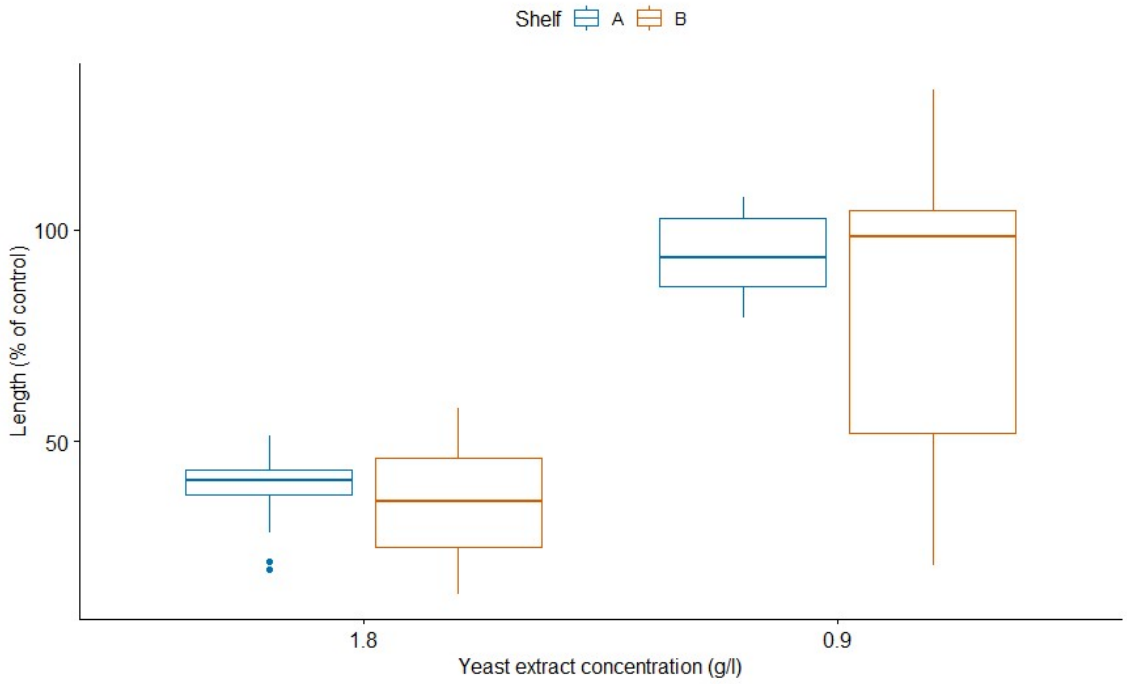

Figure 12. The difference between the different shelves A and B used in the growth room. The Col-0 col.B1 line root lengths with yeast extract, divided by the average control lengths shown as percentages. The experiment was performed only once without biological repeats, N = 14-24. Statistical tests used were two-way ANOVA for unbalanced design (Type III tests) and Tukey at the confidence level of 95 %.

In order to assess the sensitivity of the assay to possible small changes in light and other conditions, sets of seedlings were grown on two different shelves

(Figure 12). Two-way ANOVA showed that only the effect of the treatment is significant (p-value 5,968e-14, significance level 99,9 %) and not the shelf used for the experiments. Tukey post hoc tests at the confidence level 95 % support this conclusion (p-value 0,9113214 for the concentration 1,8 g/l and 0,3323741 for the concentration 0,9 g/l).

Clara Sanchez-Rodrigues gave a presentation (the 29th International Conference on Arabidopsis Research 25.-29.6.2018 in Turku) in which she stated that growing plants on buffered media can mask the growth inhibition caused by MAMPs. Media used in our assays up to this point contained the buffer, MES. Based on this, the effect of MES buffer in media was tested and the plants grown on media with MES over many different concentrations of yeast extract had longer roots than the plants grown on identical yeast concentrations without MES (Figure 13).

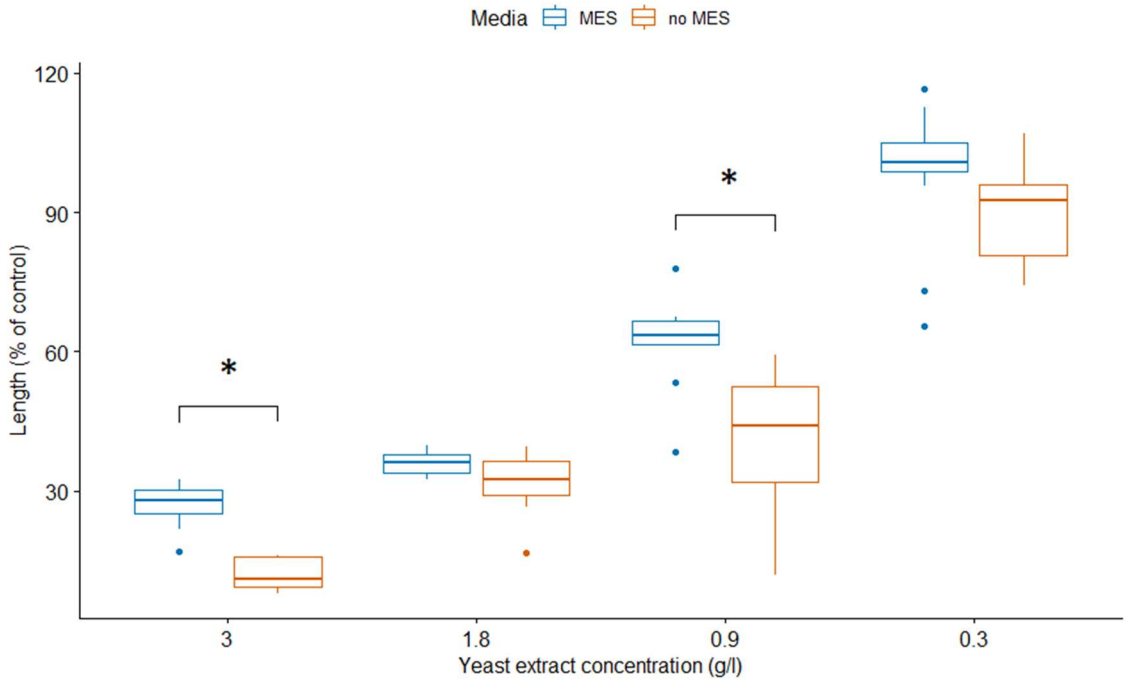

Figure 13. The difference between media with and without MES. The Col-0 col.B1 line root lengths with yeast extract, divided by the average control lengths shown as percentages. The experiment was performed only once without biological repeats. N = 9-15. The statistical tests used two-way ANOVA for unbalanced design (Type III tests) and Tukey at the confidence level of 95 %.

The two-way ANOVA showed that there is a significant difference (p-value 0,0006713) between the media with MES and the media without MES. Based on Tukey, the length difference is statistically significant at the concentrations 3 g/l (p-value 0,0148522) and 0,9 g/l (p-value 0,0000560), but not with the concentrations 1,8 g/l (p-value 0,9739892) and 0,3 g/l (p-value 0,2835207). This suggested that in our assay MAMP inhibition of root growth was greater in media without buffer.

The result for the concentrations 0,9 g/l is in line with the presentation by Clara Sanchez-Rodrigues (the 29th International Conference on Arabidopsis Research 25.-29.6.2018 in Turku) and therefore, leaving MES out of the media for the rest of the experiments is justified.

5.5.2 Testing co-receptors as potential positive controls

PRRs require co-receptors for their function and co-receptors are known to participate in many different PRR complexes. So, co-receptor mutants might also work as potential positive controls for the reverse genetics root assay as they may be insensitive to the the MAMPS in yeast extract. Mutant lines of well-characterized co-receptors ELICITOR RECEPTOR KINASE 1 (*cerk1-2*), BAK1-LIKE 1 (*bkk1-1*), BRI1-ASSOCIATED RECEPTOR KINASE (*bak1-5*) and *bak1-5 bkk1-1 cerk1-2* triple mutant (*bbc*) were first tested with different yeast concentration (Figure 14). Two-way ANOVA showed that the genotype has a significant effect on a confidence level 99 % (p-value 0,004369).

It can be seen from the Tukey analysis that the col.B2 plants are significantly longer than the mutants at concentrations 1,8 g/l and 0,9 g/l (p-value < 0.05). However, there does not seem to be a significant length difference with the concentration 3 g/l and with the concentration 0,3 g/l.

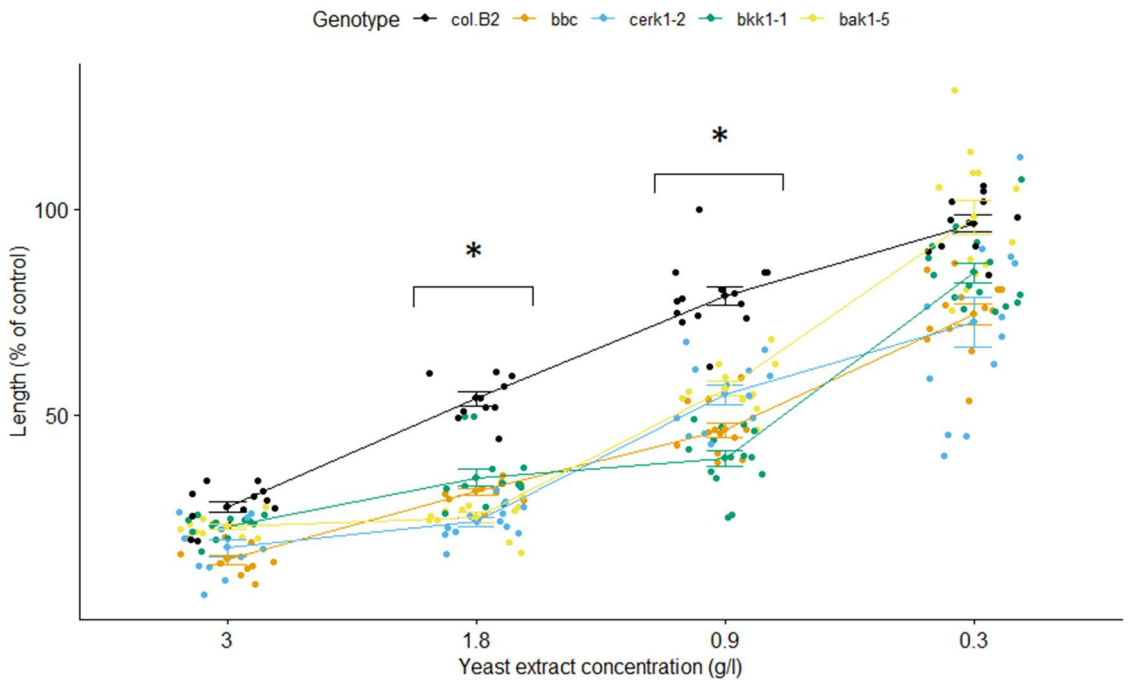

Figure 14. The potential positive control mutant root lengths with yeast extract, divided by the average control lengths shown as percentages. The experiment was done only once without biological repeats, N = 10-15. The statistical tests used were two-way ANOVA for unbalanced design (Type III tests) and Tukey at the confidence level of 95 %. Abbreviations col.B2, same age seed Col-0 control line; *cerk1-2*, ELICITOR RECEPTOR KINASE 1 mutant line; *bkk1-1*, BAK1-LIKE 1 mutant line; *bak1-5*, BRI1-ASSOCIATED RECEPTOR KINASE mutant line; *bbc, bak1-5 bkk1-1 cerk1-2* triple mutant line.

0,9 g/l was chosen as the concentration for the root assay based on the initial concentration testing (Figure 11) and the results from the co-receptor mutants tested in different concentrations (Figure 14). The four co-receptor mutants were further tested with more repeats in order to see if there is a significant difference between them and the wild type control line col.B2 (Figure 15).

The linear mixed model with repeat as a random effect shows that the genotype is statistically significant (p-value <0,0001). The result is the same when using a two-way ANOVA for unbalanced design.

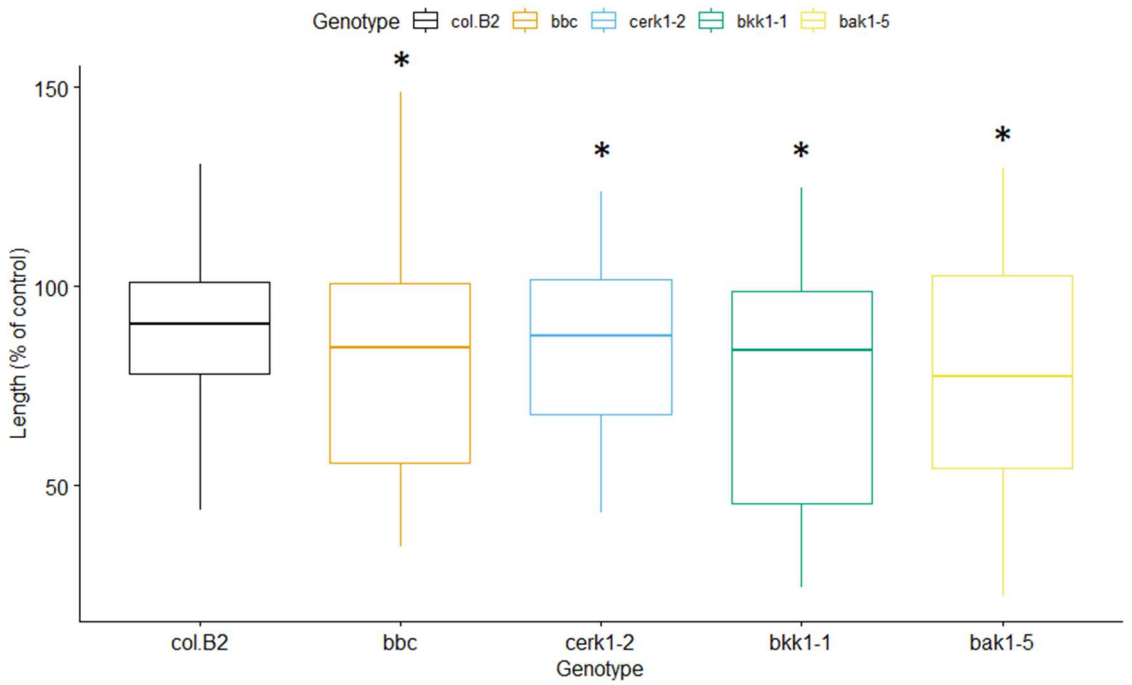

Figure 15. All three biological repeats with the 0,9 g/l concentration pooled. Root lengths with yeast extract, divided by the average control lengths shown as percentages. N = 32-45 for the pooled samples, and 9-15 for the different repeats. The statistical tests used were linear mixed model with repeat as a random effect, two-way anova for unbalanced design (Type III tests) and Tukey for the linear mixed model at the confidence level of 95 %. Abbreviations col.B2, same age seed col-0 control line; *cerk1-2*, ELICITOR RECEPTOR KINASE 1 mutant line; *bkk1-1*, BAK1-LIKE 1 mutant line; *bak1-5*, BRI1-ASSOCIATED RECEPTOR KINASE mutant line; *bbc*, *bak1-5 bkk1-1 cerk1-2* triple mutant line.

ANOVA and Tukey showed that the treated mutants have significantly shorter roots than the treated Col-0 plants (p. value <0,0001). However, the differences were small (Figure 15) when compared to a possible sensitive mutant line At4g21390.L1 (Figure 18), and the co-receptors tested might not actually be sensitive to the yeast MAMPs but for something else in the yeast extract mixture. Therefore, they might not be suitable as positive controls in similar assays and need more testing.

It must be noted that one of the *bak1-5* mutant repeats as well as one of the *cerk1-2* mutant repeats had a small contamination on the 0,9 g/l plate. However, these contaminations did not seem to have spread far enough to affect the growth

of the plants, so these plants have been included. More repeats are needed, but there were no contaminations during the first assay with different concentrations (Figure 14) that gave similar results, which supports the results obtained from these contaminated plates.

5.5.3 Mannan assay

As seen in figure 16, the results with mannan concentrations 0,3 g/l and 0,9 g/l differ from the results obtained with the yeast extract (Figures 14 and 15). Since the yeast plate experiment had three repeats and the mannan experiment was done only once, the mannan assay results have to be confirmed in future work.

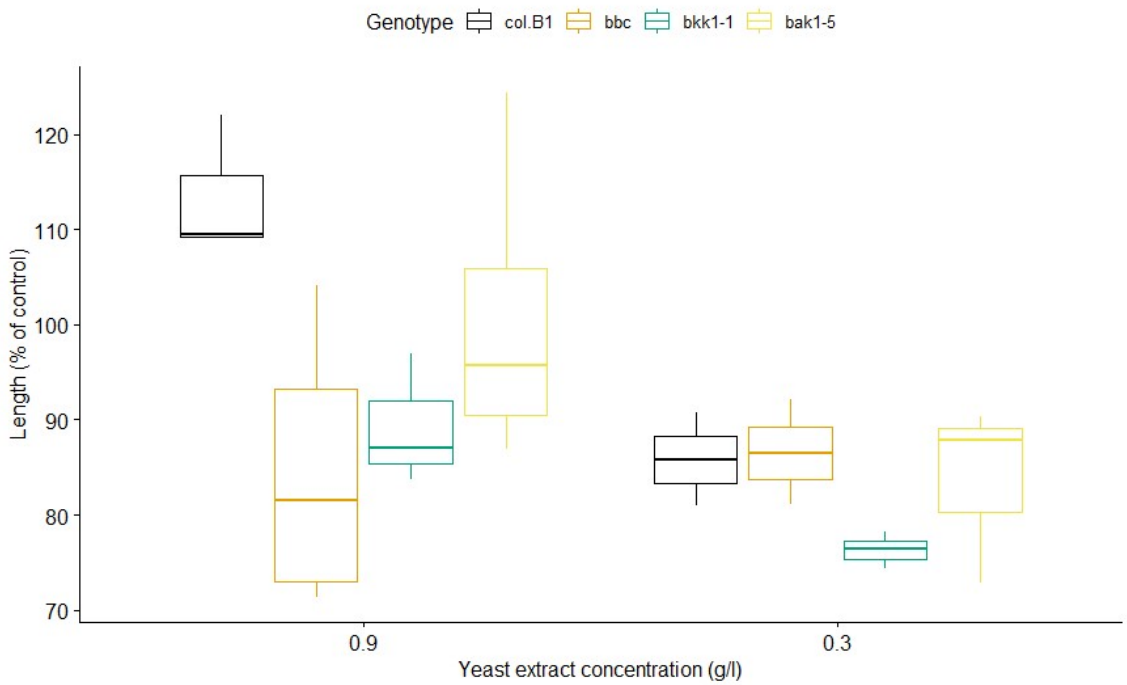

Figure 16. The mutant root lengths with mannan, divided by the average control lengths shown as percentages. The experiment was performed only once without biological repeats, N = 2-4. The experiment had significant problems yet to be solved, so no statistical tests were used to assess the acquired data.

One likely explanation for the difference is that the mannan plates had a problem with the even distribution of the mannan on the plate (Figure 17), which showed

signs of precipitation of the mannan into white dots. The problem seemed to get worse with higher mannan concentration, most likely leading to a smaller amout of effective mannan. In order to get more reliable results in the future, it is crucial that a different method will be used in order to assure that the mannan will not precipitate into small grain-like masses after being mixed with the rest of the medium.

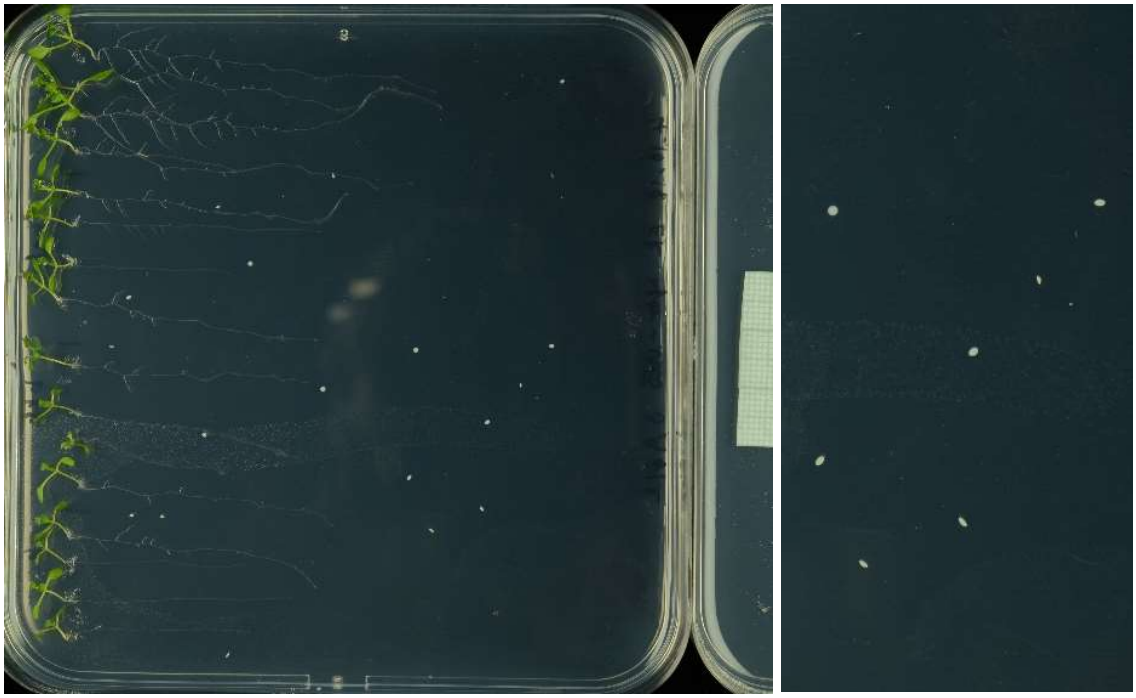

Figure 17. Precipitated masses in 0,9 g/l mannan agar plate. Another method is needed for a more even distribution of mannan in order to get reliable results.

5.5.4 Yeast extract assay with mutant lines and controls

In an effort to identify mutants sensitive or insensitive to the yeast extract, mutant lines and possible co-receptors (Table 1) were chosen based on Genevestigator results (Appendix 4) and tested with 0,9 g/l yeast extract concentration. Since the mutant lines were tested once, the results should only be used as a preliminary indicator of the possible genes involved in yeast perception before the experiments have been properly repeated with both, positive and negative controls. They can, however, give some direction when deciding which of the

potential lines show promise for future study, especially when combined to the information provided by current literature. In the future, the seeds should also be cleaned more carefully to avoid contaminations, which were a recurring problem for these experiments. Only the noncontaminated results are presented.

Since different T-DNA insertion lines are known to have variable phenotypes depending on the site of mutation that can occasionally lead to false mutants with residual expression or even increased transcript levels of the target gene tought to have lost gene function (Wang et al. 2015), all of the mutants should be inspected for expression in order to draw conclusions about their involvement in pathogen resistance. The mutation sites of each mutant tested can be found from the materials and methods (Table 1).

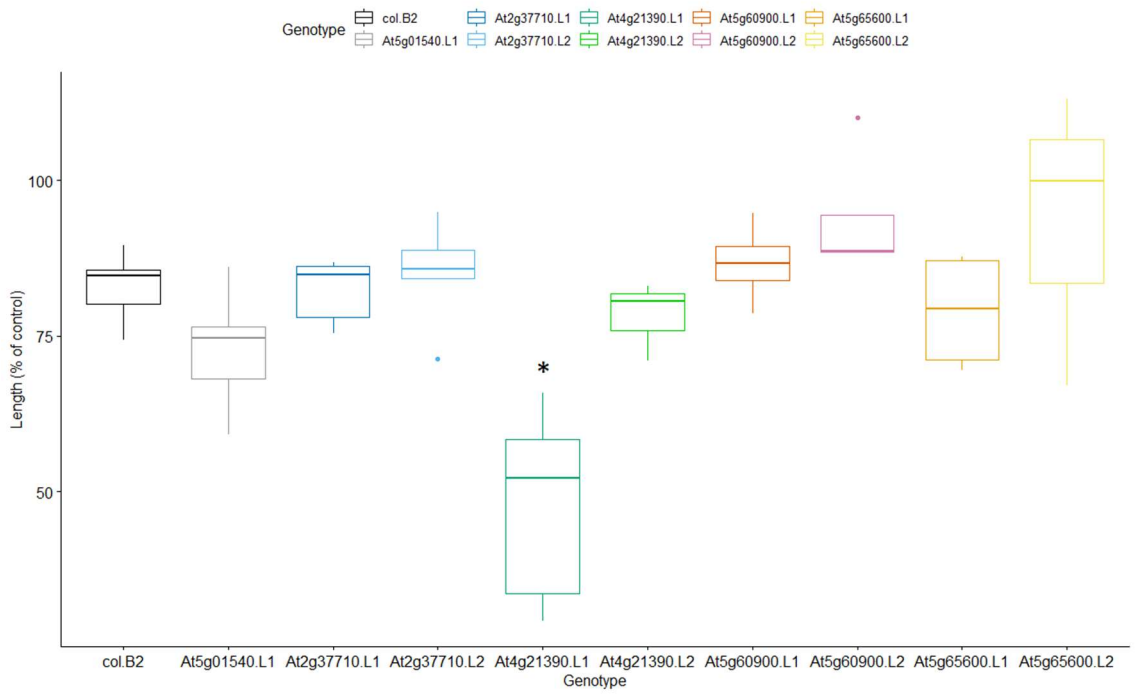

Figure 18. The batch one mutant root lengths with yeast extract, divided by the average control lengths shown as percentages. The experiment was performed only once without biological repeats. N = 3-5. The statistical tests used were one-way ANOVA and Tukey at the confidence level of 95 %.

For the first batch (Figure 18), one-way ANOVA and Tukey suggested that the mutant plant root lengths were significantly shorter than the lengths of the col.B2 control line for the line At4g21390.L1 (p-value 0,0004070, N = 5 for mutants and controls) but not for the other lines. However, this was not supported by the other At4g21390.L2 line (p-value 0,99998258, N = 3 for mutants and controls) and more repeats are needed to confirm the sensitivity. It shoud also be noted that the At4g21390.L1 line has a mutation in the position 5'-UTR, while At4g21390.L2 has the mutation at an intron.

All the other genes tested in the batch one also showed strong up-regulation with fungi and fungal-like pathogens in Genevestigator, but do not seem to behave significantly differently to the Col-0 control line. Genes At5g65600 (LecRK92), At5g60900 (RLK1) and At2g37710 (LecRK41) do not have any other genes of their gene family in close proximity, making it less likely that the phenotype would be masked by the other genes, especially since they all had two separate T-DNA knockout lines tested that gave similar results.

Line At5g01540 (LecRK62) had only one line tested that did not seem to differ from the Col-0 line, but in Genevestigator, At5g01540 showed clear up-regulation with *Hyaloperonospora arabidopsidis*, *Sclerotinia sclerotiorum*, *Plectosphaerella cucumerina*, *Golovinomyces orontii*, *Blumeria graminis* and *Phytophtora infestans*. It is an L-type lectin receptor from the expanded gene family 2 that is a known member in of the FLS2 receptor complex (Huang et al. 2014). In addition to a role in bacterial defence, it is possible that At5g01540 might also participate in plant-yeast interactions. It should be noted that genes At5g01550 (LecRK63) and At5g01560 (LecRK64) are situated in close proximity, making redundancy and gene linkage more likely, especially since At5g01550 also shows up-regulation with fungi and fungal-like pathogens in Genevestigator.

ANOVA and Tukey were also used to compare treated plant lengths to the control plant lengths within each separate mutant line to see, if some of the mutants appeared to be blind to the yeast extract. The length difference between the control plants and treated plants seemed to be statistically insignificant for the lines At2g37710.L1 (p-value 0,3856002), At2g37710.L2 (p-value 0,8042034),

At4g21390.L2 (p-value 0,3347282), At5g60900.L1 (p-value 0,9405383), At5g60900.L2 (p-value 0,9999982), At5g65600.L1 (p-value 0,4051513), At5g65600.L2 (p-value 0,9999992) and col.B2 (p-value 0,5696634). The difference appears to be significant only for lines At4g21390.L1 (p-value 0,0000000) and At5g01540.L1 (p-value 0,0053506). More repeats are needed before drawing any conclusions because of the the inconsistency of the significance of the difference between the treated and control plants for the Col-0 control line in different batches. Lines At4g21390.L1 (B120) and At5g01540.L1 (LecRK-VI.2) might still be good candidates for MAMP-insensitive mutants since Genevestigator (Appendix 4) shows regulatory changes for both when treated with fungi or fungal-like MAMPs.

At4g21390.L1 (also known as B120 or AtG-LecRK-IV.2) is a mutant line that belongs to the G-type lectins. It has an S-locus glycoprotein, plasminogen apple nematode, transmembrane and kinase domains (Teixeira et al. 2018). Genevestigator (Appendix 4) showed the B120 gene to be clearly up-regulated with *Sclerotinia sclerotiorum*, *Plectosphaerella cucumerina*, *Phytophtora infestans* and *Blumeria graminis*.

At4g21390 is situated in the fourth *Arabidopsis* chromosome in the proximity (Appendix 3, Figure 1) of another G-type lectin of the same expanded gene family one with a similar domain composition, At4g21380 (G-LecRK-VI.3 also known as SD18), which Genevestigator results also indicated to be a highly potential yeast receptor (Appendix 4). Unfortunately we were not able to acquire homozygous At4g21380 mutant lines for this thesis. Because of the proximity of the two genes, it is possible that gene linkage or redundancy mask the effect of the mutation or that the other line might be mutant for both of these genes and therefore, show an effect when the other does not.

It should also be noted that At4g21380 is the sister gene of At1g65790 (G-LecRK-VI.1 also known as SD17) (Teixeira et al. 2018) of the same expanded gene family 1 (Appendix 3, Figure 1) that has a similar domain structure and had up-regulated expression with several fungi and fungal-like MAMPs in Genevestigator (Appendix 4). At1g65790 is situated in a big gene cluster and the genotype used

in the Genevestigator experiments was a mutant also for the nearby At1g65800 (G-LecRK-VI.3 also known as SD16) that has a similar domain structure, making it another gene of interest for future experiments. We were not able to acquire homozygous mutant lines of these two genes for this thesis.

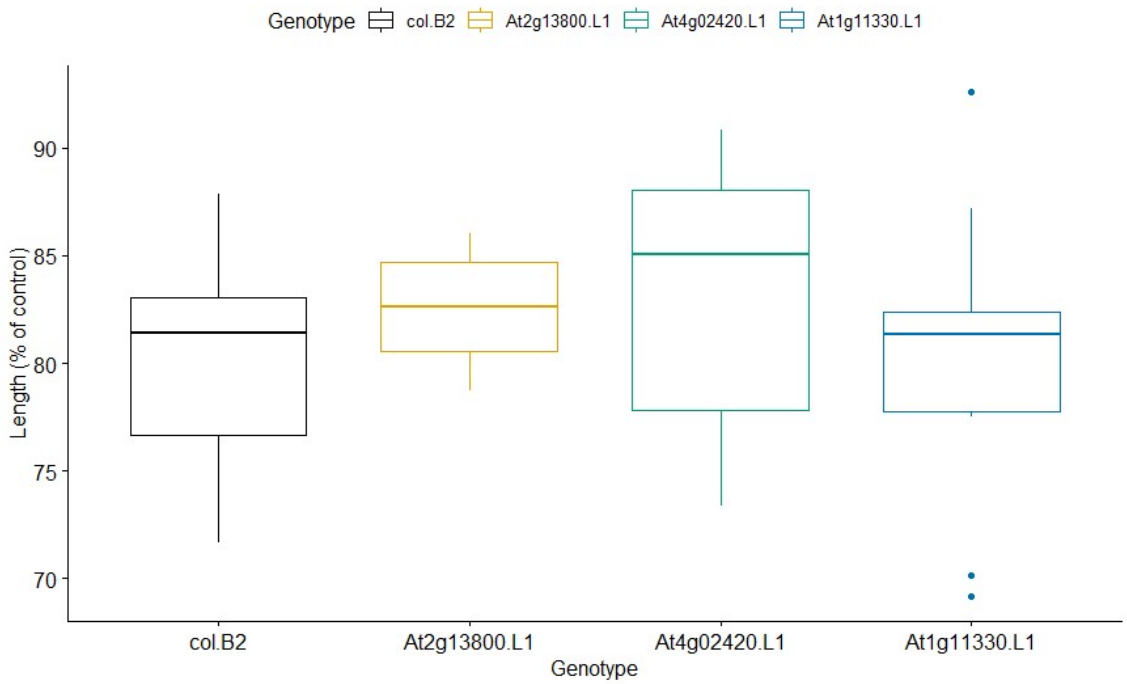

Figure 19. The batch two mutant root lengths with yeast extract, divided by the average control lengths shown as percentages. The test was performed only once without biological repeats. N = 7-10. The statistical tests used were one-way ANOVA and Tukey at the confidence level 95 %.

None of the genotypes tested in batch two seem to behave significantly differently from the Col-0 wild type when grown on a 0,9 g/l yeast agar (Figure 19). When the root lengths of the treated plants were compared to those of the untreated plants of each separate plant line, the length differences appeared to be significant for all the lines tested. Hence, it seems that lines At1g11330.L1 (p-value 0,0000015), At2g13800.L1 (p-value 0,0055397), At4g02420.L1 (p-value 0,0008427) are all behaving like the col.B2 control line (p-value 0,0001351).

These results suggest that these genes might not be putative yeast receptor candidates, but no conclusions should be arrived at before more mutant lines of the same gene are available and several repeats have been made. Control line col.B2 had no significant length difference between the controls and the treated plants in batch one but showed significant difference in batch two, giving strong indication about the unreliability of these preliminary results.

It is possible that this screen might not be ideal for the genes tested in the batch two since At1g11330 is close to the genes At1g11280, At1g11300, At1g11340, At1g11350 (SD113) and At1g11410. At1g11280 was not investigated with Genevestigator since it doesn't seem to be present in birch and At1g11300 and At1g11340 don't seem to be up- or downregulated (Appendix 4). However, At1g11350 is slightly up-regulated and At1g11410 is strongly down-regulated (Appendix 4), so there is a possibility that the mutant phenotype is masked by these other genes in the same gene family 1. At4g02420 (LecRK44) is in close proximity of the gene At4g02410 (LecRK43) of the same gene family two that also shows up-regulation (Appendix 4). At2g13800 (serk5) belongs to the SERKs (Somatic embryogenesis receptor-like kinases) that act as co-receptors for several LRR-RKs (Chinchilla et al. 2007) and was hence tested to see if it might also be significant for the plant-yeast interactions.

None of the genotypes tested in batch three seem to behave significantly differently from the Col-0 wild type when grown on a 0,9 g/l yeast agar (Figure 20). Anova and Tukey were also used to compare treated plant lengths to the control plant lengths within each separate plant line to see, if some of the mutants appeared to be blind to the yeast extract. The difference appears to be significant for the lines At1g11350.L1 (p-value 0,0098035) and At1g11350.L2 (p-value 0,0201642), At2g19130.L1 (p-value 0,0289050). There seems to be no significant difference for lines At5g01550.L1 (p-value 0,07455465) and col.B2 (p-value 0,1387539). More repeats are needed before drawing any conclusions because of the the inconsistency of the significance of the difference between the treated and control plants for the Col-0 control line.

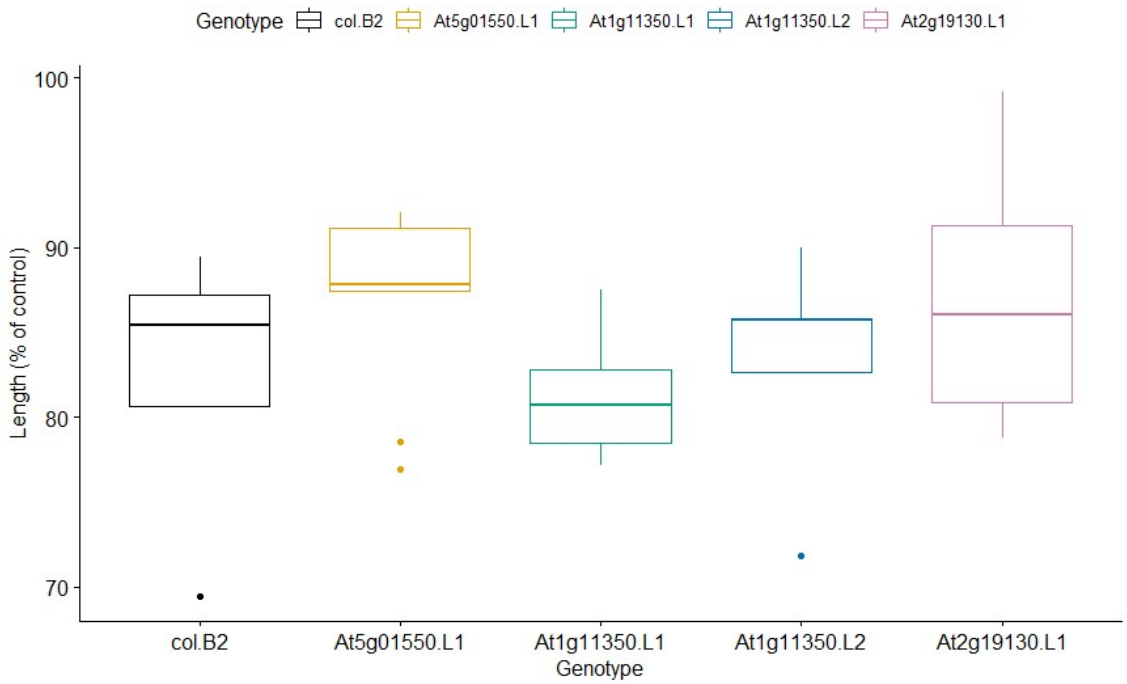

Figure 20. The batch three mutant root lengths with yeast extract, divided by the average control lengths shown as percentages. The experiment was performed only once. N = 4-9. The statistical tests used were one-way ANOVA and Tukey at the confidence level of 95 %.

At2g19130 doesn't have genes from the same expanded family in close proximity and more repeats with several different mutant lines should be performed in order to discard it as a putative yeast receptor. At1g11350 (SD113) is close to five other genes of the same gene family, including At1g11330 that was tested in the batch two. This makes a clear phenotype unlikely, especially since both At1g11350 mutant lines seem to behave similarly to the wild type. At5g01550 (LecRK63) is close to the genes At5g01540 and At5g01560. At5g01540 mutant was tested in the first batch and there appeared to be no difference in growth compared to the wild-type Col-0 plants despite the strong up-regulation seen in Genevestigator (Appendix 4), which supports the possibility of gene redundancy or linkage.

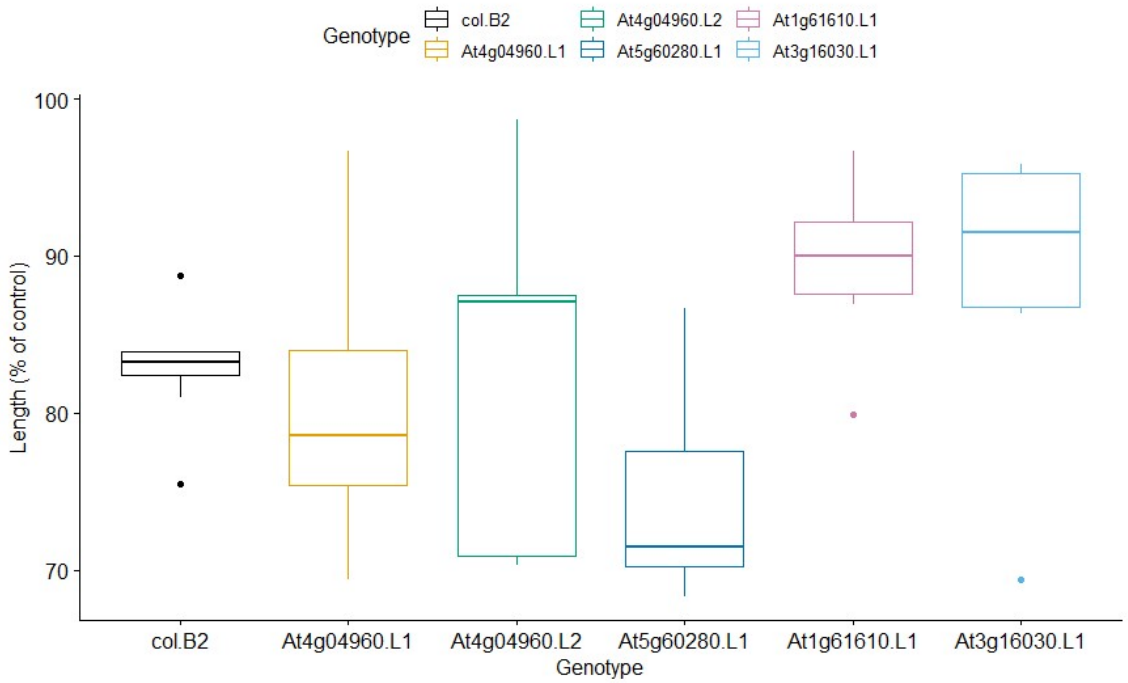

Figure 21. The batch four mutant root lengths with yeast extract, divided by the average control lengths shown as percentages. The experiment was done only once. N = 4-8. The statistical tests used were one-way ANOVA and Tukey at the confidence level of 95 %.

Based on one-way ANOVA, none of the genotypes tested in batch four (Figure 21) seems to behave significantly differently from the Col-0 wild type when grown on a 0,9 g/l yeast agar. ANOVA and Tukey were also used to compare treated plant lengths to the control plant lengths within each separate plant line to see, if some of the mutants appeared to be blind to the yeast extract. The difference appears to be significant for the lines At5g60280.L1 (p-value 0,0000054) and col.B2 (p-value 0,0060509). There seems to be no significant difference for lines At1g61610.L1 (p-value 0,5742498), At3g16030.L1 (p-value 0,3177623), At4g04960.L1 (p-value 0,1751445) and At4g04960.L2 (p-value 0,2780072). This could mean that these four lines are blind to the yeast extract, but more experiments are needed to confirm, especially since the difference was significant for the Col-0 control line in batches two and four, but not in batches one and three.

At3g16030 (CES101) and At4g04960 (LecRK71) have no other genes of the same expanded gene family near (Appendix 3, Figures 1 and 2), making growth inhibition more likely if the genes are indeed yeast-receptors. Therefore, the genes should be tested with more repeats and different mutant lines. Unfortunately, At1g61610 and At5g60280 (LecRK18) are both right next to several genes of their respective expanded gene families (Appendix 3, Figures 1 and 2) and might therefore not exhibit a clear phenotype in this type of screens.

6 CONCLUSIONS

The purpose of this thesis was to establish and apply laboratory screening methods that can help to recognise from phenotype previously unknown yeast receptors present in the plant *Arabidopsis thaliana*. Bioinformatics analysis was used to narrow down the vast amout of possible receptor candidates for wet laboratory methods. In the future, the potential mutants will go through several biological repeats of the screening assays developed for this thesis and later infected with different yeast pathogens to screen for enhanced susceptability.

The root assay method was improved by leaving out the buffering MES from the media, giving a more robust root growth inhibition by the MAMPs. The co-receptor mutant lines *bbc*, *bak1-5*, *cerk1-2* and *bkk1-1* show some promise as possible positive controls for similar root assays but might be sensitive to something else than yeast MAMPs in the yeast extract and not participate in yeast-plant interactions. On the other hand, the developmentally futher advanced insensitive mutants from the forward genetics assays and the sensitive mutant line, At4g21390.L1, from the reverse genetics root assay might potentially be used as controls after further testing. The concentration 0,9 g/l appears to be appropriate for root assay screening of yeast receptors with the yeast extract but finding the right concentration for the mannan requires more experiments.

In order to continue the experiments with mannan containing media, it is necessary to solve the problem with mannan precipitation. Even if the yeast extract is cheaper than mannan and good for initial testing, it is a complex micture

that also includes several other yeast compounds, which makes it harder to know that the compound causing the root inhibition truly is a yeast MAMP. Therefore, it is necessary to carry out further testing of the mutants with the purified mannan or testing with live pathogenic yeast species for enhanced disease susceptibility phenotypes.

The forward genetics method proved to be an efficient and relatively quick way to find mutants that appear to be insensitive to the yeast extract and might therefore be able to point out genes that take part in the yeast recognition of arabidopis. These mutants should be examined further to ensure that they are indeed yeast receptor mutants and not developmental mutants.

The biggest issue for the forward genetics assay seemed to be density and more experiments are needed to find the ideal amount of seeds for visual inspection under stereo microscope without loosing too much screen efficiency. This could potentially be helped with grid dispensers or seed sowing robots that could sow the seeds more evenly. 1,8 g/l seemed to be a better concentration than 0,9 g/l for the forward genetics assays since the possible chlorosis of the seedlings is easier to detect. It is also important to look more into the sealing methods of plates since the results might be compromised if the gas exchange is not adequate, possibly masking mild phenotypes (Matuszkiewicz et al. 2019).

Gene redundancy and genetic linkage appear to be significant problems in similar screens, so the mutants should be selected carefully based on the Tair chromosome map results combined with the Genevestigator results. All lines should be sequenced to make sure that they really have only the one mutation expected. It is also known that the knock-out efficiency of T-DNA mutants can vary leading to differences between the mutant lines for the same gene and therefore, all the lines should be inspected for expression (Wang et al. 2015). For all of these reasons, these screens should only be used as indicators of the genes that might be involved in the plant-yeast interactions, but not as a mean to exclude putative yeast receptors in *Arabidopsis*.

Some putative yeast receptors to test in the future with similar assays might be At4g21380 (SD18) from expanded gene family one and genes At3g08870 (LecRK61), At3g53810 (LecRK42), At4g02410 (LecRK43), At4g28350 (LecRK72), At4g29050 (LecRK59) and At5g01540 (LecRK62) from gene family two. These genes were clearly up- or downregulated with fungal or fungus-like pathogens, are all found in silver birch and *Arabidopsis* and are either separate from the other genes of the same gene family or have the maximum of two other genes near them. Lines At3g08870, At3g53810, At4g28350, At4g29050 and At5g01540 were used in the forward genetics pools, so it is possible that they are already included in some of the mutants collected. Known plant resistance co-receptors like SoBir1 (Van der Burgh et al. 2019) should also be tested in order to illuminate, if the plant-yeast interactions rely on the same co-receptors used with other pathogens or if the yeast receptors have co-receptors of their own.

Both methods used, forward and reverse genetics, suggest some putative pattern recognition receptors that might be involved in the recognition of yeast specific microbe associated molecular patterns. As these methods will continue to be improved further, they might prove to be usefull tools in the research of plant-yeast interactions.

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9 APPENDICES

Appendix 1: Plant lines used in this thesis

AGI code	Gene name	Involved in	Mutant name	Line(s)
At5g60900	RLK1	protein amino acid phosphorylation	rlk1.L1	SALK_084958C
At5g60900	RLK1	protein amino acid phosphorylation	rlk1.L2	SALK_146545C
At5g10530	LECRK91	defence response to bacteria and oomycetes, protein phosphorylation, positive regulation of cell death and hydrogen peroxide metabolic process	lecrk91.L1	SALK_042414C
At5g10530	LECRK91	defence response to bacteria and oomycetes, protein phosphorylation, positive regulation of cell death and hydrogen peroxide metabolic process	lecrk91.L2	SALK_149005C
At5g65600	LECRK92	defence response to bacteria and oomycetes, systemic acquired resistance (SA), protein phosphorylation, positive regulation of cell death and hydrogen peroxide metabolic process	lecrk92.L1	SALK_111817C
At5g65600	LECRK92	defence response to bacteria and oomycetes, systemic acquired resistance (SA), protein phosphorylation, positive regulation of cell death and hydrogen peroxide metabolic process	lecrk92.L2	SALK_140495C
At1g11340	RKS1	protein amino acid phosphorylation, recognition of pollen	rks1.L1	SALK_050988C
At3g16030	CES101	Innate immune response, protein phosphorylation, response to fungus	ces101.L1	SALK_136842C

At4g21380	SD18	protein amino acid phosphorylation, recognition of pollen	sd18.L1	SALK_109125C
At4g21380	SD18	protein amino acid phosphorylation, recognition of pollen	sd18.L2	SALK_001986C
At4g27290	G-LecRK-VI.4	protein amino acid phosphorylation, recognition of pollen	g-lecrk64.L1	SALK_067606C
At4g27290	G-LecRK-VI.4	protein amino acid phosphorylation, recognition of pollen	g-lecrk64.L2	SALK_129987C
At1g11300	EGM1	protein amino acid phosphorylation, recognition of pollen	egm1.L1	SALK_058300C
At1g11330	G-LecRK-V.3	protein amino acid phosphorylation, recognition of pollen	g-lecrk53.L1	SALK_143489C
At1g11350	SD113	protein phosphorylation and autophosphorylation	sd113.L1	SALK_099776C
At1g11350	SD113	protein phosphorylation and autophosphorylation	sd113.L2	SALK_026338C
At1g61610	G-LecRK-IV.2	protein amino acid phosphorylation, recognition of pollen	g-lecrk42.L1	SALK_201319C
At4g21390	B120	protein amino acid phosphorylation, recognition of pollen	b120.L1	SALK_099394C
At4g21390	B120	protein amino acid phosphorylation, recognition of pollen	b120.L2	SALK_147351C
At2g19130	G-LecRK-I.2	protein amino acid phosphorylation, recognition of pollen	g-lecrk12.L1	SALK_000051C
At2g37710	LECRK41	defence response to bacteria and oomycetes, protein phosphorylation, response to salicylic acid	lecrk41.L1	SALK_019496C
At2g37710	LECRK41	defence response to bacteria and oomycetes, protein phosphorylation, response to salicylic acid	lecrk41.L2	SALK_069646C
At3g08870	LECRK61	defence response to bacteria and	lecrk61.L1	SALK_058958C

		oomycetes, protein phosphorylation		
At3g45330	LECR11	defense response to fungi, bacteria and oomycetes, protein phosphorylation	lecrk11.L1	SALK_052123C
At3g45410	LECRK13	cellular response to salt stress and ethylene stimulus, protein phosphorylation, defense to oomycetes, fungi and bacteria	lecrk13.L1	SALK_087804C
At3g53810	LECRK42	defense response to bacteria and oomycetes, pollen development, protein phosphorylation	lecrk42.L1	SALK_119402C
At4g02420	LECRK44	defense response to bacteria and oomycetes, protein phosphorylation	lecrk44.L1	SALK_128001C
At4g29050	LECRK59	defense response to bacteria and oomycetes, protein phosphorylation	lecrk59.L1	SAIL_917_A03
At4g29050	LECRK59	defense response to bacteria and oomycetes, protein phosphorylation	lecrk59.L2	SAIL_73_D07
At5g01550	LECRK63	abscisic acid mediated signaling pathway, protein phosphorylation, seed germination, defense response to bacteria and oomycetes	lecrk63.L1	SALK_108000C
At5g01560	LECRK64	abscisic acid mediated signaling pathway, protein phosphorylation, defense response to bacteria and oomycetes, seed germination	lecrk64.L1	SAIL_114_F10
At5g01560	LECRK64	abscisic acid mediated signaling pathway, protein phosphorylation, defense response to bacteria and oomycetes, seed germination	lecrk64.2	SAIL_170_F02

At5g60280	LECRK18	defense response to bacteria, fungi and oomycetes, protein autophosphorylation	lecrk18.L1	SALK_147846C
At5g60300	LECRK19	defense response to bacteria and oomycetes, cellular response to ATP, focal adhesion assembly, response to wounding, protein phosphorylation, regulation of JA mediated signaling pathway	lecrk19.L1	SALK_039426C
At5g60300	LECRK19	defense response to bacteria and oomycetes, cellular response to ATP, focal adhesion assembly, response to wounding, protein phosphorylation, regulation of JA mediated signaling pathway	lecrk19.L2	SALK_024581
At4g04960	LECRK71	protein phosphorylation, defense response to bacteria and oomycetes	lecrk71.L1	SALK_093876C
At4g04960	LECRK71	protein phosphorylation, defense response to bacteria and oomycetes	lecrk71.L2	SALK_051149C
At4g28350	LECRK72	defense response to bacteria and oomycetes, protein phosphorylation	lecrk72.L1	SALK_141841C
At1g51800	IOS1	defense response to oomycetes, fungi and bacteria, protein phosphorylation, negative regulation of abscisic acid-activated signaling pathway	ios1-1	Zimmerli mutant
At1g51800	IOS1	defense response to oomycetes, fungi and bacteria, protein phosphorylation, negative regulation of abscisic acid-activated signaling pathway	ios1-2	Zimmerli mutant

At1g51800	IOS1	defense response to oomycetes, fungi and bacteria, protein phosphorylation, negative regulation of abscisic acid-activated signaling pathway	ios1-3	Zimmerli mutant
At1g51800	IOS1	defense response to oomycetes, fungi and bacteria, protein phosphorylation, negative regulation of abscisic acid-activated signaling pathway	IOS OE1	Zimmerli overexperssion mutant
At1g51800	IOS1	defense response to oomycetes, fungi and bacteria, protein phosphorylation, negative regulation of abscisic acid-activated signaling pathway	IOS OE3	Zimmerli overexperssion mutant
At5g01540	LecRK-VI.2	defense response to oomycetes and bacteria, protein phosphorylation, pathogen-associated molecular pattern dependent induction by symbiont of host innate immune response, seed germination, response to abscisic acid	lecrk-vi.2-1	Zimmerli mutant
At4g33430, At2g13790, At3g21630	BAK1 BKK1 CERK1	bak1-5 bkk1-1 cerk1-2 triple mutant	bbc	Zipfel triple mutant
At4g33430	BAK1	cell death, defense response to bacteria, fungi and oomycetes, brassinosteroid mediated signaling pathway	bak1-5	Zipfel mutant
At2g13790	BKK1	cell death, leaf senescence, response to chitin, brassinosteroid mediated signaling pathway, regulation of seedling development, protein phosphorylation	bkk1-1	Zipfel mutant

At3g21630	CERK1	defense response to fungi and bacteria, protein phosphorylation, protein autophosphorylation	cerk1-2	Zipfel mutant
At1g52060	JAL9	mannose binding	jal9.L1	SALK_151007C
At1g52060	JAL9	unknown, has mannose-binding lectin domain	jal9.L2	SALK_108616C
At1g52070	JAL10	unknown, has mannose-binding lectin domain	jal10	SALK_125442
At1g71830	SERK1	pollen maturation, protein phosphorylation, microsporogenesis, floral organ abscission, embryo development, golgi organization, brassinosteroid mediated signaling pathway	serk1-1	Butenko mutant
At1g34210	SERK2	microsporogenesis, brassinosteroid mediated signaling pathway, pollen maturation, protein phosphorylation	serk2-1	Butenko mutant
At4g33430	SERK3	serk 3-1 = bak1-1 cell death, defense response to bacteria, fungi and oomycetes, brassinosteroid mediated signaling pathway	serk 3-1	Butenko mutant
At2g13790	SERK4	serk 4-1 = bkk1-1 cell death, leaf senescence, response to chitin, brassinosteroid mediated signaling pathway, regulation of seedling development, protein phosphorylation	serk4-1	Butenko mutant
At2g13800	SERK5	signal transduction, protein phosphorylation	serk5-1	Butenko mutant
At1g71830, At4g33430	SERK1 SERK3	serk1-1 and serk3-1 double mutant	serk1-1 serk3-1	Butenko mutant
	WT old	older Col-0 wild type for control	col.B1	Wang WT

	WT new	Col-0 wild type for control, same age as the propagated seeds from other lines, propagated from the Wang Col-0 seeds	col.B2	Wang WT
At2g32680	RLP23	defense responce to bacteria, fungi and oomycetes, signal transduction	rlp23	Overmyer mutant
At2g39660	BIK1	pathogen-associated molecular pattern dependent induction by symbiont of host innate immune response, defense response to fungi, protein autophosphorylation	bik1_1	Overmyer mutant
At5g46330	FLS2	defense response to fungi, protein phosphorylation, regulation of anion channel activity, receptor-mediated endocytosis, transmembrane receptor protein tyrosine kinase signaling pathway	fls2	Kimura mutant
At3g55550	LecRK-S.4	defense response to bacteria and oomycetes, protein phosphorylation	S.4-1	Bouwmeester mutant
At3g08870	LecRK-VI.1	defense response to bacteria and oomycetes, protein phosphorylation	VI.1	Bouwmeester mutant
At3g53810	LecRK-IV.2	defense response to bacteria and oomycetes, protein phosphorylation, pollen development	IV.2	Bouwmeester mutant
At5g01560	LecRK-VI.4	abscisic acid-activated signaling pathway, seed germination, defense response to bacteria and oomycetes, protein phosphorylation	VI.4-1	Bouwmeester mutant
At5g60270	LecRK-I.7	defense response to bacteria and oomycetes, protein phosphorylation	I.7	Bouwmeester mutant

At1g15530	LecRK-S.1	defense response to bacteria and oomycetes, protein phosphorylation	S.1-2	Bouwmeester mutant
At5g01540	LecRK-VI.2	response to abscisic acid, defense response to bacteria and oomycetes, protein phosphorylation	VI.2-1	Bouwmeester mutant
At5g01540	LecRK-VI.2	response to abscisic acid, defense response to bacteria and oomycetes, protein phosphorylation	VI 2-2	Bouwmeester mutant
At4g02420	LecRK-IV.4	defense response to bacteria and oomycetes, protein phosphorylation	IV 4-1	Bouwmeester mutant

L, line acquired for this thesis; B, batch

The information about the processes that the gene product is involved in are collected from the NCBI website https://www.ncbi.nlm.nih.gov/ (National Center for Biotechnology Information, U.S. National Library of Medicine, 8600 Rockville Pike, Bethesda MD, 20894 USA). All names are an internal designation to this study and might not correspond to previous studies.

GNA-related lectins or G-type (G-LecRK) lectins are named according to the classification system used by Teixeira et al. (2018) and the L-type lectin receptor kinases (L-LecRKs) are named based on the classification system by Bouwmeester and Govers (2009). Whenever available, the name used in Genevestigator has been chosen to ensure easy comparison of gene regulation with different fungi and fungal-like pathogens.

Appendix 2: Primers used in this study for genotyping PCR

Gene specific primers				
AGI	Gene	SALK line	Forward (LP)	Reverse (RP)
AT1G52050	Mannose-binding lectin superfamily protein	SALK_121544	TCTCCAACATG CAAACTCATG	ATTATCATTCGCA CCGTCATC
AT1G52050	Mannose-binding lectin superfamily protein	SALK_121640	ATCGTCAATTG GCAAATTCTG	TCATCTTTTGTTT GTCGTAGGC
At1g52070	Mannose-binding lectin superfamily protein	SALK_125442	GCCTTAAAACA AGATGAAGTTC G	ACGACAAATCTG GTACGATGC
At1g52070	Mannose-binding lectin superfamily protein	SALK_125443	TGAAAGATTCA ACTGGGTTGG	TGTTGTAGGTTCC CTCCACTG
At5g60900	RLK1	SALK_146545	TGACGAGGTTC TATGGTCGAG	ATCACCATACTCA TTGCTCGG
At3g45430	LecRK-I.5	SALK_024369	GGTTCTTTCTT GGTCCCAAAG	ATTAACCGGGTG AATCAAACC
At5g60300	LecRK-I.9	SALK_024581	TTCAGACATCT CATGCTCACG	TGCAGTTGACAAA TGCTTCAG
At1g61610	AtG-LecRK-IV.2	SALK_014012	TCCAAGCTTGT TCTCTTCAGC	CTTGAGGTTTTGG ATTAGGCC
At3g45420	LecRK-I.4	SALK_029148	AACTGATGTGT ATGCCTTCGG	CACTTTTCTTGGC CAACTTTG
T-DNA primer				
lba.1	TGGTTCACGTAGTGGGCCATCG			

The primers were designed on the SIGnAL website at http://signal.salk.edu and ordered from Sigma-Aldrich Finland Oy (Espoo, Finland).

Appendix 3: Tair chromosome map results

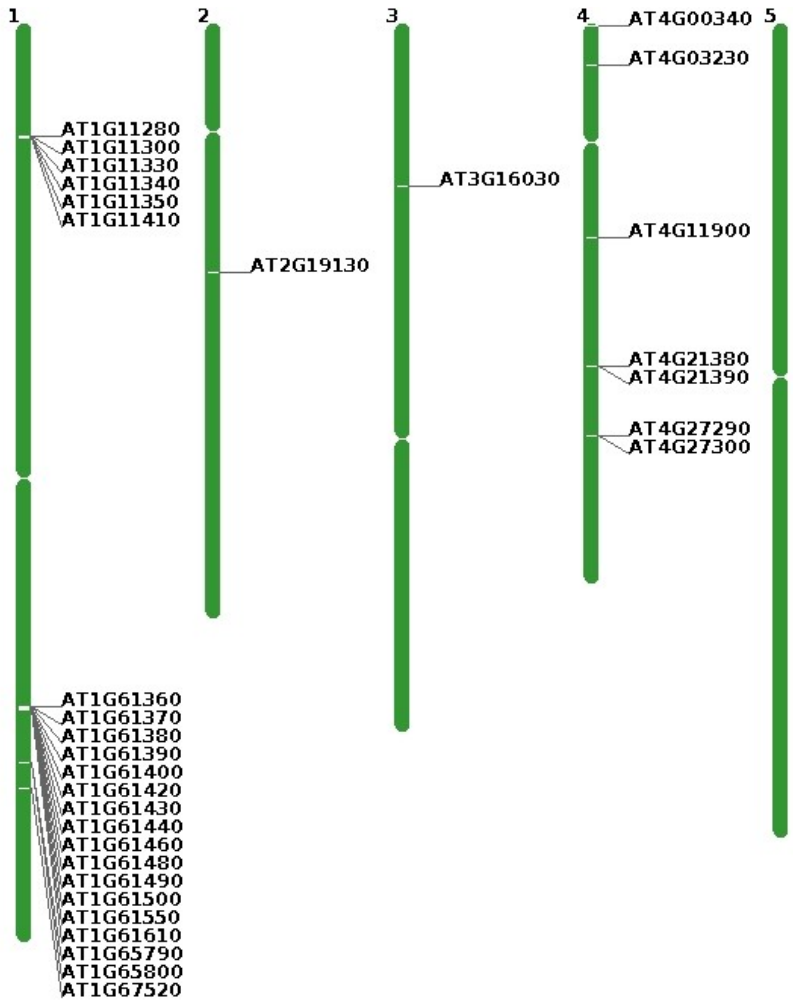

Figure 1. Tair chromosome map of the G-type expanded gene family 1. While some of the genes are clearly separate from the other genes of the family, many are situated in clusters. These genes might be problematic for the screens because of genetic linkage and redundancy.

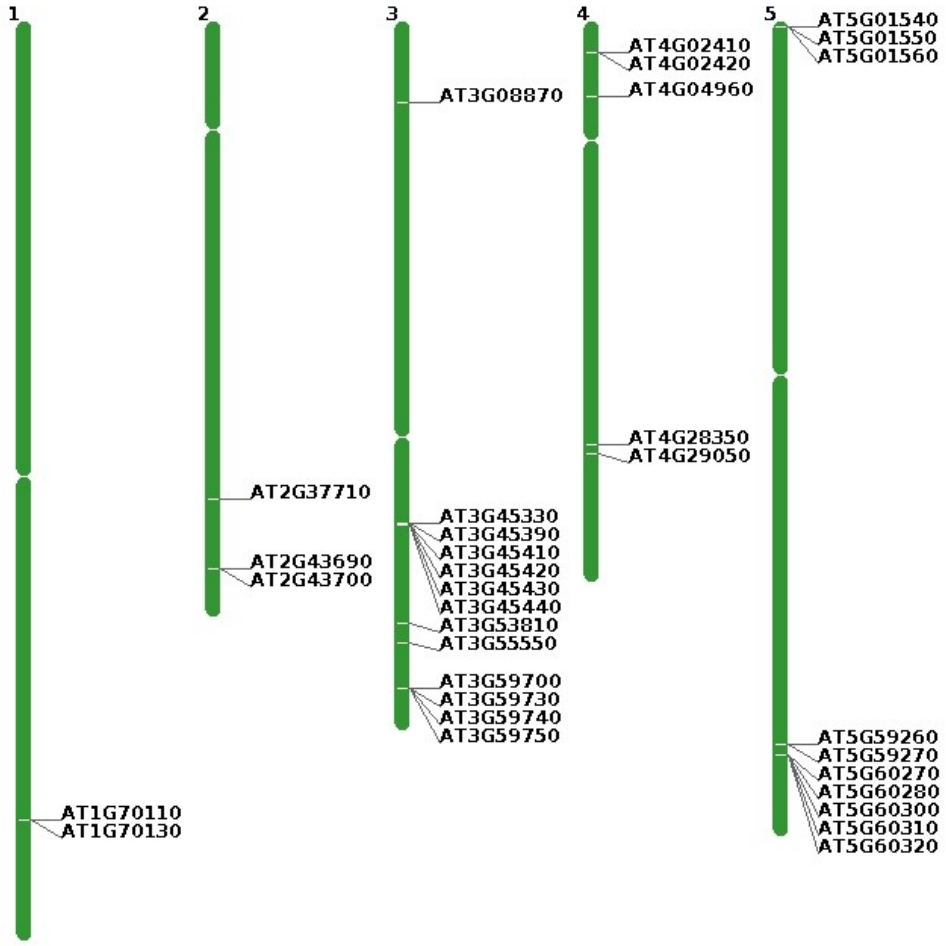

Figure 2. Tair chromosome map of the L-type expanded gene family two. Only a few genes are clearly separate from the other genes of the family while many are situated in clusters. These genes might be problematic for the screens because of genetic linkage and redundancy.

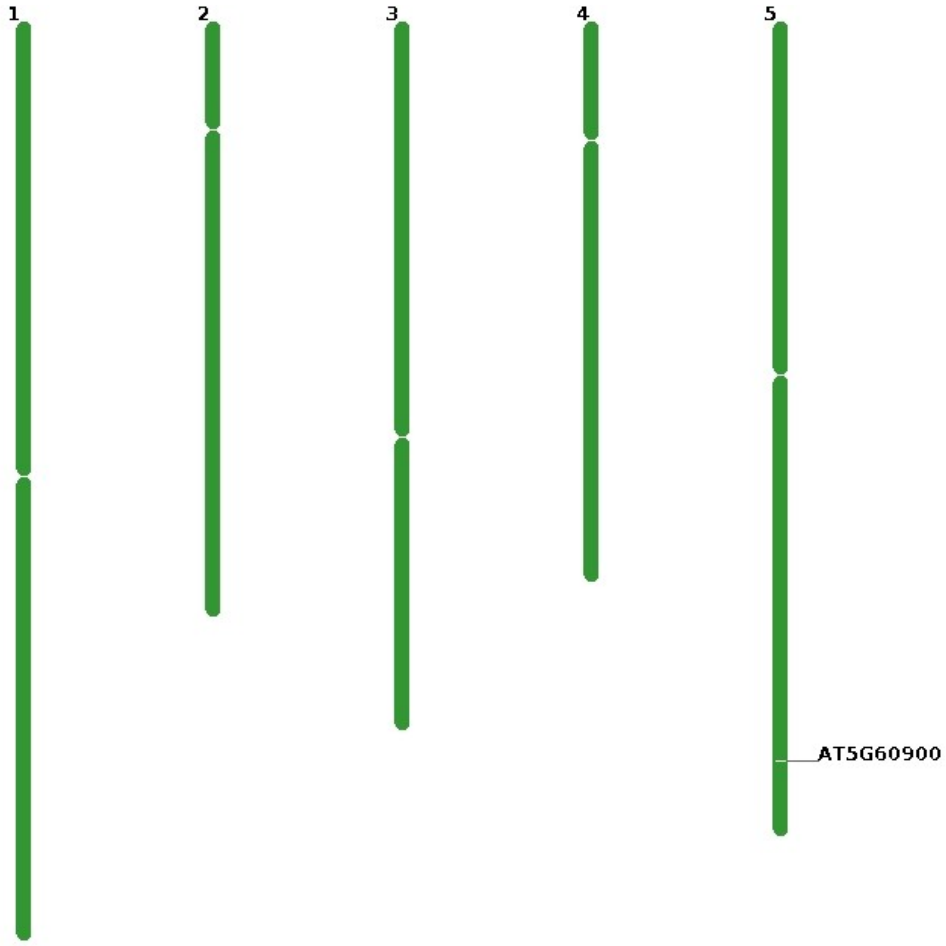

Figure 3. Expanded G-type gene family 3 has only one gene present in *Arabidopsis*. This makes it ideal for screening.

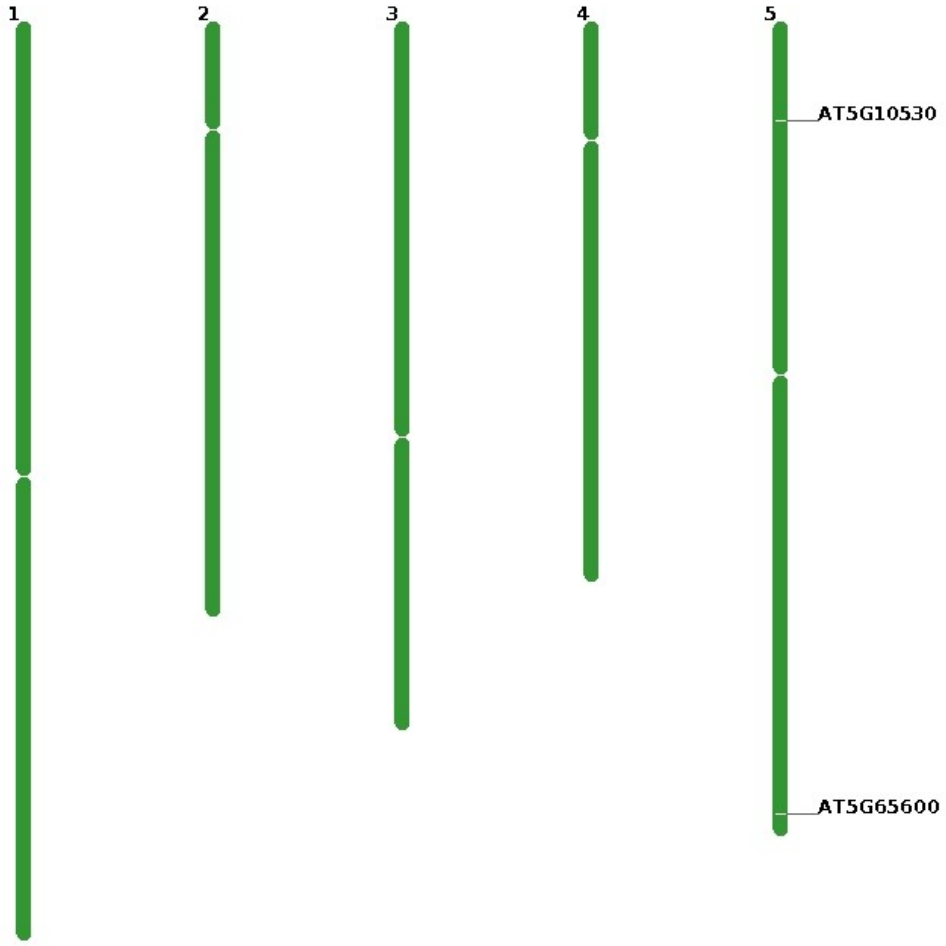

Figure 4. Expanded L-type gene family 4 has only two genes in *Arabidopsis*. These genes are in the same chromosome but far apart, making genetic linkage and redundancy unlikely.

Appendix 4: Genevestigator results